

Japan's seismic tragedy at Kobe

The most destructive Japanese earthquake since that in Tokyo in 1923 will have implications far outside Japan as attention there turns to the search for safety in a seismically hazardous place.

THE tragic aftermath of last week's earthquake at Kobe, southwest of Tokyo, will cast a long shadow, and not only in Japan. There, in what is arguably the best-ordered society in the world, it will be an affront to everyday contentment that nearly 5,000 people have been killed and 250,000 people made homeless by a mere geological event. Like Manhattan, but on a vastly larger scale, the Pacific seaboard of Japan is a manmade dwelling place built with ingenuity and a breathtaking regard for appearance. It will surprise nobody that the survivors will be in shock for many months to come. *Nature* hereby sends its sympathy to all those in Japan who may read this and what follows.

It is also no surprise that the Tokyo stock market has taken a tumble in the past few days. The immediate economic disruption of the earthquake is something to be reckoned with, but the money-bags no doubt also calculate that Japan will now embark on a programme of public works intended further to protect its people against seismic danger. Even now-rich Japan will be hard-pressed both to strengthen buildings against the next big earthquake and to continue its investment in the industries that have made it an economic powerhouse in the past four decades. But nobody should underestimate the resourcefulness of those who have built the manmade industrial complex of Japan in such seismically hostile territory.

The lesson of this earthquake, as of all others, is that seismic waves themselves do not kill people in large numbers. But buildings and other components of the built environment do that when they collapse, or when they are set alight by ruptured gas supplies. Last week's destruction shows how huge the destruction can be. But Japan's familiarity with earthquakes and their destructive effects is also illustrated by the general use of the word 'tsunami' for the tidal waves caused by submarine earthquakes, often at great distance. Japan is no stranger to events like that at Kobe last week.

What is to be done? More seismology is not the immediate answer, although there is some evidence that the rescue services would have been more quickly in action if there had been a more rapid seismic assessment of the magnitude of the Kobe earthquake and of its character and location. In Japan, where even the railways run like clockwork, there will be shame that the rescue services seem not to have been as quickly off the mark as expectations of efficiency require. Rescue can save lives and (by firefighting) even property. Japan, with its vulnerability to earthquakes, needs a careful reassessment of what can be done to bring its vast resources

of energy and equipment to bear on the next big earthquake, whenever that will be. (No doubt they will also be dusting off the plans for rescue in California after last week's experience.)

Next comes civil engineering. Experience in all seismic regions, but notably in Japan and California, has amply shown what benefits can accrue from buildings designed against earthquakes. The whole world, not just Japan, needs a careful survey of the buildings surviving from last week and an understanding of the mechanical reasons for their survival. That will be the easy part. Devising new construction codes and enforcing them will no doubt follow, with all the horrendous cost implications they must entail. But the retro-construction of existing buildings and motorway routes may prove to be less expensive than starting all over again. Japan may be in for a period in which demolition outpaces new construction.

But are these not poor substitutes for the prize that seismology should deliver to Japan — an effective seismic prediction service? That, sadly, is an impossible dream for the present, and may remain so for many years and even forever. Japan's own publicly funded prediction service has a poor reputation and no record of even meagre success. The search for premonitory signs of earthquakes (such as methane emission as well as seismic signals), conducted with immense if fitful enthusiasm, has so far yielded nothing substantial. And carefully instrumented seismic regions, as are some sections of the San Andreas fault in California, are unlikely in the near future to yield predictions of the kind on which rescue services can take action. Until that is possible, predictions (given unavoidable uncertainty) may do more harm than good.

So is there nothing seismology can do? Luckily, the tale is not entirely gloomy. Eventually, measurements of seismic strain in the neighbourhood of active faults may help to tune construction codes more accurately to regional long-term seismic risks. As things are, neither the data nor the understanding exist for such an exercise. Given the huge extra costs of earthquake-proof construction, the case for collecting data and for their intelligent analysis is overwhelming. But seismology must be careful not to over-sell the promise of these long-term studies. At some level, a degree of fatalism is required of governments and the people they represent. Occasional catastrophes are a part of the price of living on the surface of a tectonically active planet. Pity Kobe nevertheless.



Republican critics step up pressure on technology assessment office

Washington. The US Congress's Office of Technology Assessment (OTA) is edging closer to the brink of closure by Republicans, who have dominated the House of Representatives since last November's elections. Last week Ron Packard (Republican, California), who chairs the House of Representatives appropriations subcommittee that funds the OTA, joined Connie Mack (Republican, Florida), his Senate counterpart, in supporting its elimination.

House Republicans who support the OTA, led by Amo Houghton (Republican, New York), who will chair its management board if it survives, hope to have a meeting with Mack this week to plead for the survival of the \$22-million, 200-strong agency, which assesses science and technology issues for the Congress.

But Mack's staff say he has made up his mind to pursue the closure of the OTA, following the endorsement of that course of action at a closed meeting of the 53 Republicans in the 100-strong Senate last month. They add that at a meeting with Packard last Friday, he too supported OTA's closure.

Since its creation in 1972, the OTA has produced several hundred detailed reports for Congress on everything from nuclear proliferation to the state of the US contact-lens industry. In doing so, it established the principle that an effective legislature needs its own source of advice on science and technology matters, in order to handle political decisions involving increasing technical complexity. Under the leadership of Jack Gibbons — now President Bill Clinton's science adviser — from 1980 to 1993, the OTA became a model widely imitated in other industrial countries.

Lewis Branscomb of Harvard University, a member of OTA's advisory council, says that scientists and engineers should support the OTA against the current threat. "It took 20 years to build this into a highly trusted agency," he says. "I hope the scientific community will rally behind it as a valuable resource that the country needs."

The advisory council met Houghton and OTA officials in Washington two weeks ago, and advised the officials to collect examples of the OTA's work that would attract the attention of members of Congress who are unfamiliar with it.

But, given the OTA's penchant for detailed and thorough reports that present options without issuing clear-cut recommendations, this may prove a difficult task. A leaflet being circulated by the OTA, for example, shows that it has saved the government \$14 million a year by saying that a treatment of Alzheimer's disease needed no government regulation; \$368 million on computers for the social security department; and — fancifully — \$60 billion on the Synthetic Fuel Corporation, a pet project of Jimmy Carter, the former president.

Robert Walker (Republican, Pennsylvania), who chairs the House Science Committee, has expressed support for a smaller agency that would produce advice in less than the 18 months that OTA takes for its major reports. Roger Herdman, OTA's director, says the agency is ready to speed up and reduce its size.

But Herdman says the agency has already reduced its management by 30 per cent over the past two years, and refuses to accept that it should offer to cut a third of its staff. He says that Congress plans to cut its

support agencies by 10 per cent, and that the OTA should be treated likewise.

Herdman, a 61-year-old former paediatrician and public health official, joined the OTA as head of health and life sciences in 1983. He succeeded Gibbons last May after some well-known outsiders, including Maxine Singer, president of the Carnegie Institution, turned down the job.

Some of OTA's supporters in Congress say that it has lost its high public profile and some of its best people under Herdman. But he says that a smaller management structure, with seven out of nine division heads newly promoted, means that the agency is maintaining its intellectual edge.

OTA's present troubles began last February when Mack attacked its reports for their lack of focus on technology. In response, Herdman drew up new rules specifying each study's technological content. But a senior member of Mack's staff dismisses this as a "minor managerial change".

Mack feels his concerns were ignored when he was a minority senator; now they cannot be. Hence the recommendation of a task force, chaired by Mack and Senator Pete Domenici (Republican, New Mexico), to close the agency. Ancient grudges — for example over OTA's more partisan early days — are not thought to have played much of a role in the Republican Conference's acceptance of that.

Instead, an agency that does its work for an officer corps of congressional committee heads may be paying a price for its obscurity in the ranks. "If you did a market research study on OTA's efficacy in Congress, the response would be 'OT what?'," says one Republican member of staff. "And it's not bad PR; it is a question of the usefulness of the product. I can't name an OTA study in five years that has played an integral part in legislative activities."

Neil Harl, professor of economics at Iowa State University and chairman until last month of the OTA advisory council, says that the closure of the OTA "would be a tragic outcome". Those fighting to save it will argue that it is uniquely equipped to help frame laws on very complex issues, such as the patenting of the human genome.

But they face an uphill task. The prevalent view in Congress today is that it does not need an exhaustive, peer-reviewed study before deciding to reject unnecessary schemes such as the Synthetic Fuel Corporation. The reports being read in Congress this year emanate from corporate-funded think-tanks such as the Cato Institute: they may lack objectivity — but they come quickly to the point, and free of charge to the taxpayer.

Colin Macilwain

How the earthquake struck Japan

A map prepared by the US Geological Survey (right) shows the major plate boundaries, marked by yellow lines, in the area where the Kobe earthquake struck on 17 January. The map also shows significant earthquakes that have occurred between 1961 and 1994, the size of a dot being proportional to the magnitude of the earthquake, and its colour indicating its depth.

Last week's earthquake, denoted by a star, occurred on a strike-slip fault branching off a larger strike-slip fault known as the Median Tectonic Line. Rupture zones are indicated by red lines. (See also page 273.)

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US Geological Survey

Glaxo bid 'reflects changing patterns of drug research'

London. Pharmaceutical research in Britain — including that in new fields such as cell and molecular biology — is heading for a shake-out following Monday's announcement by Glaxo, already one of the world's largest drug companies, that it is making a bid for its smaller rival, Wellcome plc.

Glaxo officials say that one of the main aims of the proposed take-over is to enable the new joint company, which it intends to call Glaxo Wellcome, to achieve the economies of scale needed to continue to pursue research leading to innovative new products in a highly competitive global environment.

If the deal goes ahead, it will also benefit biomedical research directly through the fact that Glaxo would acquire the 40 per cent of the shares



Sykes: job losses are 'inevitable'.

in Wellcome that are currently owned by the Wellcome Trust, the body set up on the death of the company's founder, Henry Wellcome.

The trust is Britain's largest private source of research funding, operating on a scale comparable to the Howard Hughes Medical Institute in the United States — and with a research budget almost as large as that of the Medical Research Council. Last Sunday, it agreed in principle to accept Glaxo's offer, which values shares in Wellcome at 48 per cent higher than they were previously being quoted on the open market.

But the merger, which is now being considered by the board of Wellcome, would also lead to substantial job losses in both research and manufacturing. The activities of the two companies already overlap in a number of fields — for example, the treatment of cardiovascular disease and diseases of the central nervous system.

There is also concern in some quarters that the sheer size of the combined venture, which would become the largest private company in Britain, could lead it to play an excessively dominant role in determining future directions of pharmaceutical research.

Describing the reasons behind Glaxo's bid, which values Wellcome's share capital at £8.9 billion (\$14 billion), Richard Sykes, the company's former research director who took over as chief executive two years ago, said that one powerful motivating factor had been a recognition of "a change in the way that drugs are going to be developed in the future".

A statement issued by the company ex-

plained that while expertise based on traditional disciplines such as biochemistry and pharmacology continue to be applied in research, "increasing opportunities are being presented by newer but more costly technologies as a result of profound developments in cellular and molecular biology".

Sykes admitted that the planned 'rationalization' and streamlining of the research teams of the two companies, one of the main factors being used to generate support for the take-over among shareholders, will inevitably lead to job losses among researchers.

"Both our companies have excellent research pedigrees and an international reputation," he said. "In those area of research, such as cardiovascular disease, where each has people working, we will be looking to see which are the best programmes of the two, and then putting these together in the new company."

But, aware of the opposition that Glaxo's proposals are likely to generate from labour unions, Sykes also argued that increasing pressure on costs in the pharmaceutical industry world-wide meant that "there will be job losses, whether these two companies come together or not".

He pointed out that, even though Glaxo spends over £1 billion a year in research and development — more than any other British company — it still has only 3.9 per cent of the global drug market. "There has to be a change," Sykes said. "It cannot carry on that way, and we want to be in the lead in the process of consolidation."

In choosing to bid for a company with a complementary portfolio of pharmaceutical products, Glaxo has eschewed the strategy of some of its rivals — for example, the US company Merck Sharpe and Doehme — who have opted for 'vertical integration' by merging with companies directly involved in patient care and disease management.

Sykes said that Glaxo had looked at this strategy in exploring other possible mergers, but had decided in the end "to stick to our basic objective of producing better medicines". He also said that the Wellcome Trust — which for legal reasons is declining to comment on Glaxo's bid — has indicated that it will "take a significant investment in the new group".

Indeed, the terms of the proposed acquisition, which would involve paying for Wellcome shares with a combination of cash and 'New Glaxo shares', will result in the trust holding 4.7 per cent of the joint company's share capital. But it will also leave the trust free to invest its endowment capital more flexibly than in the past.

David Dickson

UK group to explore potential benefits of 'foresight' exercise

London. As British government officials complete the final reports of the 15 panels engaged in its wide-ranging "technology foresight" exercise, preparations are already well advanced for a number of projects designed to analyse the results as soon as they are published and turn them into 'user friendly' form.

Last week, for example, the Centre for the Exploitation of Science and Technology (CEST), created in 1987 as a bridge between the research and industrial communities, announced the launch of a new fund to support the centre's research in fields ranging from transport to the life sciences.

Its first project will be to show companies how to gain a competitive advantage by applying to their corporate strategies the output of the foresight exercise launched in the wake of the 1993 white paper on science, and intended to generate a consensus on the most promising lines of new technological developments.

"British companies need to learn to hunt in packs if they are to exploit foresight's activities", says Bob Whelan, CEST's chief executive, adding that if Britain fails to make good use of the conclusions of the exercise, "we will have provided free-market intelligence to our competitors which they will have then take up and use in competition against us".

CEST hopes to raise £5 million towards its research activities over the next five years, part of which will come from subscriptions and donations from the centre's members, which include both large corporations and government departments.

The new fund is being seen in some quarters as a response to issues raised in an external review of the report activities of the centre, carried out at the end of 1993. This commended much of the centre's work, but said that many members felt that it needed to do more to justify their subscriptions (see *Nature* 368, 8; 1994).

● David Hunt, Britain's minister for science, announced on Tuesday that the government is to set up a committee to advise it on efforts to develop the public understanding of science. The new committee will be chaired by Sir Arnold Wolfendale, the Astronomer Royal, and will be made up of both practising scientists and science journalists.

Addressing a select committee of the House of Lords, Hunt also said that a project officer will be appointed to look into proposals for improving the conditions under which microbial culture collections are kept, and to recommend to Hunt's Office of Science and Technology the costs and benefits of the steps needed to achieve this goal. □

AIDS activists fail to block 'reasonable pricing' reform

Washington. A government task force agreed last week to back negotiations that are expected to lead to the National Institutes of Health (NIH) dropping the controversial 'reasonable pricing' clause in agreements between intramural researchers and industry.

Under pressure from critics on both these sides, who argue that the clause is an impediment to collaboration, the NIH is negotiating with the Department of Health and Human Services (DHHS) about alternatives; a decision to drop the clause is expected within the next few weeks.

In a majority vote, the National Task Force on AIDS Drug Development, agreed that the NIH should continue its current negotiations. But this led to a noisy protest by the AIDS activist group ACT-UP, which had urged the task force to press NIH to strengthen the clause. The group bases its case on the excessive costs of the anti-AIDS drug AZT, which was partly developed with federal funds.

The members of the task force, which is responsible for removing barriers to drug development for AIDS, include both Harold Varmus, director of the NIH, and David Kessler, head of the Food and Drug Administration. It is often a forum for policy debates — such as that on reasonable pricing — that go beyond AIDS alone.

Reasonable pricing clauses are included in the NIH's cooperative research agreements (CRADAs), introduced by Congress in 1986 to facilitate technology transfer from government agencies, and in exclusive licensing deals. CRADAs did not at first require such clauses. But NIH introduced them in 1989, following widespread outrage at the price of AZT.

NIH is the only major government agency to have such a clause, and many intramural researchers say that it prevents them from collaborating with industry. "The number of complaints from our scientists has increased during the past six months," says Sandy

Chamblee, acting director for science policy and technology transfer. Last December, members of advisory councils to three NIH institutes urged Varmus to drop the clause.

Many in industry also oppose the clause, saying that they do not trust the government's ability to decide on a 'reasonable price' for drugs, and that venture capitalists are reluctant to lend money if a company has a CRADA containing a clause allowing the government to do so.

Industry gave its opinions at a public hearing last July when the NIH hosted the first of two *ad hoc* meetings to hear views on the reasonable-pricing clause. It became clear during the meeting that NIH staff were listening favourably to industry's arguments (see *Nature* 370, 238; 1994). As a result, the NIH published a list of options at the end of last year, ranging from dropping the clause completely to making a variety of changes.

Chamblee, one of the NIH staff negotiating with the DHHS on the clause, says it is now expected to be dropped. But where the NIH has substantially reduced the risk to a pharmaceutical company involved in drug development, there is likely to be a rule that the company should offer some form of public benefit in addition to paying royalties.

For example, if a company takes on a compound that the NIH has already taken through Phase-I clinical trials, it might make price concessions or develop ways of making the compound available to those who could not otherwise afford it. Many companies already have such 'accessibility' programmes, but patient advocates claim that they do not always reach their target.

But whatever emerges from the negotiations, the NIH now appears to have accepted that reasonable-pricing clauses are a barrier to research. Varmus told last week's task-force meeting that the NIH is not a regulatory body, and needs to return to its central mission of supporting basic biomedical science.

Helen Gavaghan

Republican warns of \$35-billion cut in 1996 budget

Washington. Congress will try to cut \$35 billion from next year's discretionary, non-military budget of \$270 billion — the source of most federal funding for science — according to the chairman of the House of Representatives committee that divides up the budget pie.

"You are going to be shocked when you see how austere the 1996 budget will be," Robert Livingston (Republican, Louisiana), chair of the House Appropriations Committee, warned on Monday. Cuts on this scale would certainly come as a shock to federally funded research and development, which consumes \$36 billion, almost 15 per cent of the total.

Livingston says that many agencies and programmes will be eliminated in order to make the cut, and that the largest and most popular government science programme — the \$11 billion spent on biomedical research by the National Institutes of Health (NIH) — is "on the table" with everything else.

Proposals for such deep cuts from the House may be modified by both the Senate and the Clinton administration. But some constitutional experts argue that the House has almost unfettered — but previously untested — power to cut budgets by refusing to approve the spending of any money at all.

Livingston promises that if President Bill Clinton vetoes any of the 13 bills that make up the budget, the House will send him back an even tougher bill. If no bill is signed, Congress will appropriate nothing, obliging the President to shut down those parts of the government covered by the bill in question.

Asked whether he agrees with John Edward Porter (Republican, Illinois), chair of the House appropriations subcommittee with responsibility for the NIH, that biomedical research deserves more money, Livingston said: "There is never enough money for good, solid research that is going to help us fight disease and save lives. Everything has to be on the table, including programmes we never looked at before because we were in the minority". Livingston says that "re-examining the efficiency of NIH and the priorities of NIH is not something we'd take off the table entirely".

Livingston said the rescission package for the current 1995 fiscal year (see *Nature* 373, 179; 1994) being worked on by his committee would be modest, as President Clinton "would veto it if it were too austere". The President presents his 1996 budget proposal on February 6: the House and Senate will amend it during the spring and summer and ask the President to sign the new version in September, when the 1995 fiscal year ends and the brinkmanship begins.

Colin Macilwain

New hominid remains found in Ethiopia

London. A new adult skeleton of the recently discovered 4.4-million-year-old *Australopithecus ramidus*, the earliest known hominid, has been found at Aramis in Ethiopia.

The skeleton was unearthed by Yohannes Haile-Selassie, a member of the multinational Middle Awash Research Team led by Tim White of the University of California, Berkeley. Discoveries by the same team at Aramis during 1992 and 1993 led to the naming of the new species (see *Nature* 371, 306–312; 1994).

In contrast to the first report, which

was based on only 17 specimens, the newly found skeleton consists of more than 90 fragments which together make up about 45 per cent of a skeleton. It includes pieces of bone from the skull, arms, vertebral column, pelvis and legs.

Leg material is important; this was lacking from the original report, and will help clarify whether *Australopithecus ramidus* walked erect. But a definitive answer may take some time to arrive. The skeleton is extremely fragile, and remains encased in rock and plaster awaiting study.

Henry Gee

Kobe earthquake shatters faith in engineers

Tokyo. The collapse of multi-storey buildings, and the rupture of highways and railway lines in the earthquake in Kobe has stunned the Japanese public and left civil engineers with the embarrassing task of explaining why their designs failed to stand up to the earthquake.

The collapse of the highways in particular has caused major disruption both to the relief effort and to supply routes for industry throughout the region. Officials of the Construction Ministry continue to insist that the 250 km of expressway in Tokyo will stand up to the equivalent of the Great Kanto Earthquake of 1923 which measured 7.9 on the Richter scale. But many sections of the expressway in Tokyo are even older than that which failed in Kobe and have a similar design.

As the dust clears, it is evident that much of the structural failure can be traced to old, less-stringent building codes that applied before 1980. But some modern buildings also failed. On the bright side, the many new multi-storey buildings on Kobe's manmade Port Island stood up to the earthquake, despite extensive liquefaction of the reclaimed land on which they stand.

A year ago, when the Northridge earthquake levelled highways in Los Angeles in the United States, Japanese civil engineers who visited the disaster site returned home and confidently reassured the Japanese public that Japan's highways were built to higher standards and could withstand such an earthquake. Yet although the ground motions that shook Kobe were probably no stronger than those that occurred at Northridge (see below), the Hanshin expressway, a major artery of Japan's road system, collapsed in several places.

"I am shocked," said Manabu Ito, a civil

engineer at Saitama University, on TV Asahi the morning after the earthquake as he looked at television pictures of a 600-metre section of the Hanshin expressway that had toppled on its side (see right). "I could not imagine this would happen".

But George Housner, professor of engineering at the California Institute of Technology and an expert in earthquake engineering, says that he is not surprised by the highway's collapse. The highway was built 25 years ago and did not incorporate modern principles of ductile design, pioneered in California in the 1970s but not brought into Japanese building codes until 1980.

Inspection at the site by civil engineers tends to bear out Housner's view. The highway's vertical support pillars, which sheared, are made of concrete and have vertical reinforcement rods running through them that are supported by horizontal hoops. Under the old building code, the hoops were spaced at intervals of about 30 cm. But under the new code they have to be every 10 cm.

Many of the multi-storey buildings in Kobe that collapsed were made of reinforced concrete with the same large spacing of hoops, suggesting this is a factor in their collapse. But one or two more modern buildings with 10-cm spaced reinforcing have also collapsed.

Another factor is that the highway on top of the support pillars in the long section that failed is made of reinforced concrete. The immediately adjacent section made of steel did not collapse. The reinforced concrete weighs twice as much as the steel, creating a top-heavy structure that put enormous strain on the vertical pillars when subjected to the strong horizontal forces of the earthquake. Steel sections of the highway elsewhere also collapsed, but not so extensively,

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Two-pillar construction may have avoided Hanshin expressway collapse.

and only at joints.

Tsutomu Kimura, a civil engineer and president of the Tokyo Institute of Technology, says there is an inherent weakness in the T-shaped support pillars because the single pillars have "no redundancy". If one pillar fails, the whole highway on top collapses.

A two-pillar structure would be safer, but Japan has opted for the one-pillar design in many places because of lack of space, because highways usually have to be built over existing roads, and because prohibitively high land prices prevent the government from buying up the necessary land for double supports.

Moves to reinforce the T-shaped pillars with steel casing have been going on for several years. But fewer than half of the hundreds of pillars in the Tokyo and Osaka-Kobe region that need reinforcement have been treated because of budgetary constraints.

Kobe has large tracts of reclaimed land along its waterfront, but this does not appear to have been a factor in the collapse of the highways, according to Kenzo Toki, a civil engineer from Kyoto University who has visited the disaster site.

In fact, the many multistorey buildings on Port Island withstood the shock, despite liquefaction of the underlying reclaimed land, because the buildings have pillars in their foundations extending to a depth of more than 30 metres to root them into solid ground underlying the reclaimed layer.

However, one tank for liquefied gas on reclaimed land in Kobe port did rupture, probably due to subsidence, and thousands of nearby residents had to be evacuated. Such tanks are another major concern in Tokyo because there are hundreds of them for petroleum, gas and chemicals, rimming Tokyo Bay on recently reclaimed land.

David Swinbanks

Striking similarities with US quake

Tokyo. The way that the ground shook during last week's Kobe earthquake appears to have been similar to that which took place during the earthquake under Northridge in Los Angeles exactly one year earlier — on 17 January 1993 — according to preliminary data from Japanese researchers.

Both earthquakes were relatively shallow, and occurred off the main plate boundary, under heavily urbanized areas. The Kobe earthquake was of greater magnitude (7.2 on the Richter scale) as opposed to 6.6 for Northridge. But, unlike Northridge, the earthquake was not centred directly under Kobe but about 20 km to the southwest, under Awaji island.

A network of 10 seismometers in the Kobe-Osaka region set up by Kenzo Toki of

Kyoto University in April 1994 recorded maximum ground velocities of 30–55 cm s⁻¹ along the fault zone that ruptured. This compares with a maximum of 170 cm s⁻¹ at Northridge.

But two of Toki's meters in the centre of the earthquake zone went off the scale at 40 cm s⁻¹ as they had been set at higher sensitivity. Furthermore, according to Toki, a seismometer operated by Osaka Gas Company in central Kobe also went off its scale at 99.9 cm s⁻¹. This indicates that the maximum velocity was at least 100 cm s⁻¹.

The peak acceleration of 818 cm s⁻² (almost equal to the acceleration of gravity) in a north-south direction, reported by the Japan Meteorological Agency, is similar to the maximum recorded in Northridge. **D. S.**

Canada to set up genome research centre in Toronto

Montreal. Canada's largest centre for human genome research and molecular medicine will be built at the Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto, through government and industry grants totalling C\$15.3 million.

Bristol-Myers Squibb will provide C\$10.3 million over five years for molecular biology research at the centre, almost doubling the C\$5.75-million it gave to the institute in 1990.

William Scott, a senior research vice-president for the company, has made it clear that the federal government's recent expansion of patent protection was a factor in the company's decision to make the new award.

The institute said that the company's contribution also helped to secure C\$5 million in funding from the Canada/Ontario Infrastructure Works programme to build the centre, to be called the Centre for Human Genome Research and Molecular Medicine. Federal, provincial and local governments will each contribute one-third of the cost of the 25,000-square-foot laboratory.

The centre's research will focus on gaining an understanding of genetic factors responsible for human development and the molecular basis — and perhaps the subsequent diagnosis and treatment — of such diseases as breast and prostate cancer, diabetes, asthma, birth defects, heart, bone and neurodegenerative diseases.

According to the institute, the centre will provide 150 full-time and permanent research positions and provide training in DNA and gene methodologies.

David Spurgeon

Brazil keeps former minister in post

São Paulo. Brazil's new president, Fernando Henrique Cardoso, who formally took office earlier this month, has decided to retain the former minister of science and technology, José Israel Vargas — a relatively unusual situation as new presidents usually change all government ministers.

Cardoso is a well-known sociologist and a former professor of the University of São Paulo, and Brazilian scientists are hoping that he will help them secure a reprieve from the hard times they have faced since the fall of the government of Fernando Collor de Mello in 1992.

Many are also optimistic about the retention of Vargas, who kept his position primarily because it is not considered a politically significant post. Vargas says he will continue to promote the technology needed to make Brazil's industries more competitive.

Under the administration of President

Soros foundation is defended against charges of spying

Moscow. Boris Saltykov, the Russian minister for science, last week came to the defence of the International Science Foundation (ISF) against charges that it is being used by the United States to spy on the activities of Russian scientists. Saltykov told a press conference in Moscow that he was planning to discuss the charges — widely seen as part of an attempt by internal security forces to restore their credibility after the Chechnia imbroglio — with other members of the Russian cabinet, in an effort to persuade them that they lacked any foundation.

The ISF was set up in 1992 to provide financial support for Russian scientists primarily through funds provided by the Hungarian-born financier George Soros. By the end of 1995, the foundation will have distributed \$125 million to about 50,000 scientists.

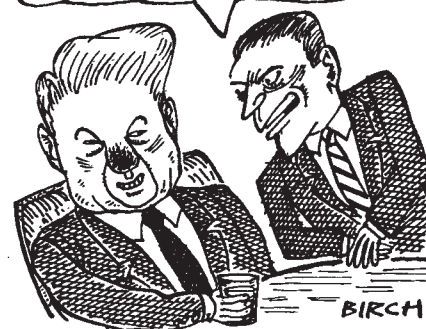
But earlier this month, the *Nezavisimaya Gazeta* ('Independent Newspaper'), published what it claimed to be excerpts from a report prepared by the federal counter-intelligence outlining how various US bodies were being used by US "special services" for carrying out "espionage and subversion on the territory of Russia".

The report pays particular attention to bodies such as the ISF, which have been collating information about the activities of Russian scientists as well as supporting collaborations with research teams in the West. "While publicly stating their aim as being to promote the development of Russian science, such bodies seek to conclude contracts with Russian scientists which result in exclu-

sive rights being ceded to their discoveries and inventions," the report says. "By doing this, Americans effectively doom the Russian science and economy to lag behind."

The report points out that the ISF used questionnaires to obtain information from individuals and research centres, in return for which it offered them research grants. "The expert analysis of American question-

And it should be easier to storm the ISF headquarters than the Chechen Presidential Palace...



naires showed that the polls conducted by the US research centres provide them with an opportunity to receive much information about the person questioned, his relatives, their employment, their superiors and other data."

ISF officials deny that they are involved in espionage, claiming that, far from attempting to undermine the strength of Russian science, their only objective is to find ways of keeping it alive and helping it to thrive.

The report is being seen by some as a bid by conservative elements within the administration of President Boris Yeltsin to discredit reformist groups (such as the so-called YABLOko faction in Parliament) which often have close links with Western-funded organizations.

But officials are still worried that, even if the report — dismissed as "naive" by the newspaper *Pravda* — cuts little ice with leading politicians and scientists, it could still have an adverse effect on administrators and researchers lower down the hierarchy, who may be reluctant to establish links with an organization held in suspicion by some parts of the Yeltsin administration.

Saltykov's intervention follows a personal request from Soros for an explanation of the status of the report.

Soros flew to Moscow last week to discuss the situation with Saltykov. At a press conference after their meeting, he said he welcomed Saltykov's assurances, and repeated his statement that the charges against the ISF were totally without foundation.

Carl Levitin

Ricardo Bonalume Neto

Italian minister faces short spell, hot seat

Rome. Giorgio Salvini, a particle physicist from the University of Rome, was last week appointed minister for universities and research in Italy's interim government. He is the fifth research minister since Antonio Ruberti left office after the general elections in spring 1992.

Like his immediate predecessors, Salvini may well be in office for only a matter of months. This is unlikely to be long enough to get to grips with the many problems faced by Italian science, particularly the lack of medium- and long-term planning of research.

Salvini's selection by the new prime minister, the banker Lamberti Dini, came as a surprise to Italian scientists. He is a respected scientist with many years of experience in research management but, at 74, he is rather old for such a difficult office.

Salvini set up the national laboratory for particle physics research at Frascati in the 1950s, and was president of the National Institute for Nuclear Physics (INFN) in the late 1960s. He also led the Italian research group that participated in Carlo Rubbia's UA1 experiment at the European Laboratory for Particle Physics (CERN), which led to the discovery of two fundamental particles, the Z and W bosons.

Since 1990, Salvini has been president of Accademia nazionale dei lincei, the Italian academy of sciences. He now takes over a ministry demoralized by nearly three years of uncertainty. Staff at the ministry look back nostalgically to the era of Ruberti, whose achievements included the introduction of a degree of autonomy into the overcentralized university system, various new research programmes, and the establishment of the Italian Space Agency (ASI).

This momentum has since been lost. Ruberti was succeeded by Sandro Fontana, who held office for 11 months until the government collapsed. He was replaced by Luigi Berlinguer, whose tenure was extremely brief, even by Italian standards: it was a matter of only hours before his PDS party (former communists) resigned from the government.

Umberto Colombo, previously head of the National Agency for New Technology, Energy and the Environment (ENEA), was then appointed minister in the interim government, headed largely by 'technocrats' rather than professional politicians, that took office up to last summer's elections.

During his year of office he sketched out a number of reforms, defining broad priorities for Italian research and proposing a steady increase in research funding from 1.6 to 2.0 per cent of gross national product, and mechanisms for increasing the efficiency with which research money is spent.

These included the introduction of conventional refereeing systems into the National Research Council (CNR), widely criticized for distributing its funds in small packets to keep everyone happy. But Colombo did not have time to see many of his proposals become reality, hoping instead that they would be endorsed by the next elected government. (He is also being tipped as a possible eventual successor to Salvini).

When Stefano Podestà, a member of Forza Italia, was appointed as research minister in Silvio Berlusconi's government last May, he appeared ready to take Colombo's proposals further. But as the government encountered growing difficulties, his actions — and the motives lying behind them — seemed to many to become confused.

Although Italy's economy could not support an immediate increase in general research funds as proposed by Colombo, Podestà's clumsy attempt to cut by a fifth the 1995 budget of ENEA, a body he regarded as inefficient, failed when parliament cut his proposals by 50 per cent.

His bill to change the system by which professors in Italy are selected has been heavily criticized in parliament, in particular for its suggestion that associate professors who have been in post for 12 years could be automatically promoted to full professor on the simple approval of an internal university committee.

Two attempts last autumn to rush through legislation to restructure the financially troubled Italian space agency ASI, and to concentrate control in the hands of a single administrator, were defeated on the grounds that parliament did not consider the issue sufficiently urgent to warrant emergency legislation (see *Nature* 372, 719; 1994).

Podestà also made himself unpopular among scientists by a decision to include a majority of orthodox Roman Catholics on the national bioethics committee. This led to the resignation of leading members of the committee (see *Nature* 373, 97; 1995).

Podestà's failures are widely seen as partly a consequence of a refusal to discuss his plans either with experts in the fields concerned or with his fellow politicians, many of whom are also academics. The lack of cross-party support for many of his proposals could have been predicted, while his successor will now inherit more immediate problems than he will have time to deal with.

One result is that hopes for a strong long-term national research policy may once again have to be shelved. "We are used to putting long-term planning aside", says a spokesman from the ministry for universities and research. "But in reality it is dangerous for Italy's future."

Alison Abbott

DNA fingers illegal trade in hawks

London. The Royal Society for the Protection of Birds (RSPB) is collaborating with the British police in using DNA fingerprinting to help prevent the theft of eggs from the nests of protected birds of prey in the United Kingdom.

The technique is being used in court cases to reveal whether the offspring of registered captive birds of prey are in fact related to their claimed parents, or have been stolen from nests in the wild and passed off as birds bred in captivity. One result has been a sudden fall in the number of such birds claimed to have been bred in captivity.

A case was due to be heard by Haverling magistrates this week (but has now been adjourned until March), in which charges against two breeders will be backed by the demonstration that, of more than 30 peregrine falcons they claim to have bred, over 20 have been shown by DNA fingerprinting to be unrelated to the 'parents'.

The prosecution — the seventh of its kind — has been brought by the Metropolitan Police, with the help of the RSPB. According to Guy Shorrock, investigations officer for the RSPB, a number of unscrupulous breeders mix eggs from captive birds with those stolen from nests in the wild.

IMAGE
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Wary predator: a falcon in the wild.

Shorrock says that the acceptance by the courts of DNA fingerprinting, which has replaced the need to catch egg thieves in the act, is making potential thieves think twice. "Genetic fingerprinting is a very powerful forensic tool and the sudden decline in captive breeding figures [for peregrine falcons and goshawks] is because it is acting as a deterrent."

The success of the technique has led the RSPB and others to demand the setting up of DNA profile databases of all rare captive birds, to help prevent theft, smuggling and nest robbing. A working group appointed by the government to review the enforcement of wildlife controls is considering whether to recommend genetic registration systems in a report it is due to present in July.

Maggie Verrall

Europe 'needs one voice on large machines'

London. France is planning to propose that European research ministers should hold an annual meeting to establish a joint position in negotiations with the United States and Japan on the financing, construction and use of future large-scale research facilities.

It is also suggesting, as the current president of the Council of Ministers, that the political status of the European Commission's Science and Technology Research Committee (CREST) should be raised, taking it out of the hands of the commission and turning it into an advisory committee to the council itself.

Both moves are intended to reinforce efforts at establishing a European-level policy for science, but without giving excessive powers to commission officials in Brussels and thus acknowledging the need to respect the concept of 'subsidiarity'. But both are also likely to be closely examined by other member states of the European Union (EU) to see whether changes being proposed by France will have a significant practical impact.

Speaking at a meeting in London last week, François Fillon, the French minister for research and higher education, said that Europe's recent decision to proceed with the construction of the Large Hadron Collider (LHC) at the European Laboratory for Particle Physics (CERN) — inviting Japan and US participation as being desirable but not essential — indicated the strength of taking a joint approach (see *Nature* 372, 713; 1994).

"The Europeans must be able to speak with one voice, when they so wish, in negotiations with Washington and Tokyo," said Fillon, delivering the annual Zuckermann lecture organized jointly by the Cabinet Office's Office of Science and Technology and the private Foundation for Science and Technology. The LHC decision, he added, had demonstrated "the resolution of the member states of CERN to move forwards, among Europeans, prior to discussions with third countries".

Facilities such as CERN and the recently created European Synchrotron Radiation Facility indicated that much had already been achieved towards creating a 'Europe of science'. But Fillon added that "much remains to be done to combine our strengths, consolidate our experience [and] launch new initiatives".

One need, he said, is to invent new institutional, financial and political forms for European scientific cooperation. "Our priority must be the scientific optimization of all projects which are necessarily undertaken by means of an agreement between European [states]," said Fillon.

Such optimization should determine the forms of cooperation, and not the reverse, he said. "Everything must be done to avoid the construction of a scientific Europe locked

into too formal an institutional approach, which creates problems rather than solving them."

When it comes to large-scale equipment that can be constructed only through global cooperation, Fillon said that Europe must be in a position to exert influence as a major player in multilateral negotiations. Ways should be found of encouraging both US and Japanese participation in European facilities without changing either their scientific or institutional objectives, as well as responding to the aspirations of the countries of Central and Eastern Europe.

Recognizing the increasing importance

IMAGE
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Fillon: seeking a
'rational realism'.

their position in such negotiations.

The proposed reform of CREST would help to turn the committee, which has recently been criticized for becoming increasingly bogged down in administrative responsibilities (see *Nature* 370, 166; 1994), into what Fillon described as a "permanent, high-level body to debate the major issues of European research".

European states "do not have a place where they can debate major issues because this is in the hands of the commission, which

of large research facilities of multilateral negotiations — particularly for future projects in fusion and space research — Fillon said that he is planning to propose to his European colleagues an annual meeting of research ministers to coordinate

is responsible for the agenda of the committee," said Fillon. "We want to clarify the situation, and to see the committee being run by whichever state also holds the presidency of the EU."

Many of Fillon's ideas coincide with those of Edith Cresson, the former French prime minister who took over this week as the new EU commissioner for research. She has already indicated a desire for a greater political involvement by member states in promoting scientific initiatives at the European level (see *Nature* 373, 183; 1995).

At the same time, while endorsing closer collaboration on the construction of research facilities, Fillon warned that basic research should be acknowledged as a field "which cannot *a priori* lend itself to a transfer of skills at European Union level" — an apparent reference to the need for each country to build up and protect its own scientific community; that "France would not be able to relinquish such a crucial factor determining its future".

He also emphasized that European-level collaboration in science has to correspond to the national interests of member states, describing this approach as a "rational realism" without which the building of a 'Europe of science' risks becoming engulfed in complicated bureaucratic procedures designed in Brussels.

"[Procedures for] the adoption of the community framework research programme and the management of invitations to tender [for contracts] are already exceptionally heavy and complex," Fillon said. "If we fail to react, the very legitimacy of such a noble ambition will be compromised in the long term in the eyes of both researchers and companies."

David Dickson

But France keeps EMBL guessing

Munich. France's approach to European-level collaboration in science (see above) is already being put to the test over its attitude towards plans by the European Molecular Biology Laboratory (EMBL) to combine two independent ventures at a single site near Rome: a concentration of molecular biology research groups, and a proposed European mouse gene repository.

Italy threatened to leave EMBL last year because it did not feel that it was receiving sufficient return on its investment. The threat was withdrawn before Christmas after the Italian government had been reassured by EMBL's eagerness to give Italy a better deal — including its agreement to place four 'regional research groups' in laboratories at Monterotondo near Rome.

EMBL is hoping that these laboratories will also be selected as the site of a mouse

gene repository, for which a consortium of national research organizations are seeking EU support through a special research infrastructure programme.

But two of the four countries involved in the application for funds — France and Britain — have still not agreed that Monterotondo is the best site. Both are apparently concerned that the negotiations between EMBL and Italy have appeared to pre-empt a decision without full discussion within the consortium.

Discussions are still taking place over who should apply for the EU funds — the deadline is in March — and who should take on the management of the repository. France, which opposes any expansion of EMBL on cost grounds, believes that management should be left to one or more national research organizations. **A. A.**

Germany plans new aid for scientists in east

Munich. Proposals for a new government research fund, intended to provide continued support for hundreds of senior east-German scientists who might otherwise lose their jobs next year when interim aid to east Germany comes to an end, are being drawn up by the Wissenschaftsrat, Germany's science council.

The fund would be limited to scientists who rely on grants for their salaries — proportionately there are more of these in the east than in the west. But the Wissenschaftsrat hopes that the proposed fund would also help to tackle the relative lack of competition for funds between researchers working in national research centres in west Germany.

The east German problem has more recent roots. After reunification in 1990, research institutes belonging to the East German Academy of Sciences were reviewed by the Wissenschaftsrat. Many were closed, and others were absorbed into the research organizations of West Germany, such as the Max Planck and Fraunhofer Societies.

But, at the Wissenschaftsrat's suggestion, the federal government also agreed to the creation of three new national research centres: the Max Delbrück Centre for Molecular Medicine in east Berlin, the Centre for Geophysics in Potsdam and the Centre for Environmental Research in Leipzig-Halle.

The government laid down conditions for the new national centres, hoping to avoid repeating past mistakes in the west. West German institutions have been criticized over the past decade for their inflexibility and variable research quality. Scientists have job security, and their research is funded fully by the government; as a result, there is no pressure to compete for outside grants with other German researchers.

The Wissenschaftsrat planned that the national research centres in the new *Länder* should rely much more heavily on short-term competitive grants, with far fewer principal investigators benefiting from permanent posts than in the west. At the Max Delbrück centre, for example, founded in 1992, it was planned that the basic budget would support 350 permanent positions, with an additional 250 posts funded by competitive grants (see *Nature* 360, 617; 1992).

Accepting that east German scientists were unlikely to succeed in obtaining significant grants immediately, the federal government created a temporary fund to supplement the basic budget of the research institutes set up in this way. In 1994 this money funded about 300 positions in the east, and in 1996 — the last transition year — around 100 will be funded.

But expectations that scientists would

generate sufficient external funding to support themselves and their research by the time this money runs out now appear to have been too ambitious.

One problem is that funding agencies such as the Deutsche Forschungsgemeinschaft (DFG) do not in general cover the salaries of principal investigators, making it almost impossible for scientists on short-term contracts to lead their own research groups. Nor is industry a likely source of increased funds, as most industrial grants are for applied research, not basic.

Competition for all grants is particularly intense at present. The budget of the DFG has not increased in real terms for the last five years. But the pool of scientists competing for grants previously intended only for west Germany has increased following reunification and a special DFG fund for east Germany, already widely considered insufficient, ran out at the end of last year.

The prospects for scientists in the east are bleak. "Unless other means of funding are found, the continuation of successful projects is threatened, and entire research groups may have to be dissolved," says Klaus Fleischmann, executive secretary of the Arbeitsgemeinschaft der Grossforschungseinrichtungen, the umbrella organization for national research centres. **Toni Feder**

Cell biology centre plans to break the mould on staffing

Paris. The Institut Curie in Paris, which has a long-running tradition of both supporting basic research and exploiting it directly to prevent, diagnose and treat cancer, is to set up a FFfr150-million (US\$28-million) cell-biology centre next year.

The creation of the centre represents an unusual experiment for French science, in that the institute plans to reserve a quarter of posts for postdoctoral researchers on short-term contracts. These researchers will be encouraged to create their own teams but take them elsewhere after five years.

The institute is situated in the Latin Quarter of Paris, combining at a single site a modern hospital — the Claudius Regaud hospital — for cancer patients, and research departments in biology, medicine, physics and chemistry. These are linked by a 'technology transfer' corridor occupied by both researchers and clinicians.

The complex was established as the Institut Radium in 1909 by the University of Paris and the Institut Pasteur, with Marie Curie heading one of the first laboratories. In 1970, the Institut Radium was merged with the Curie Foundation to form the Curie Foundation-Institut Radium, later renamed the Institut Curie.

The FFfr100-million construction costs of the new centre, due to be officially opened

in October, and its FFfr50 million of equipment, will be paid for by the institute, which is a private foundation with a budget that grew from FFfr306.5 million in 1986 to FFfr514 million in 1992. The salaries of scientists working at the centre will be mainly paid either by public research organizations, such as the Centre National de la Recherche Scientifique (CNRS) and the biomedical research agency INSERM, or by grants from other research agencies.

The new centre will add 3,500 square metres to the existing 6,000-square-metre biology department and accommodate another 150 staff in addition to the 350 at present. The Subcellular Structure and Cellular Dynamics programme will be made up of 10 research groups headed by Jean-Paul Thierry. This programme will be jointly funded by the CNRS and Institut Curie.

The centre will be headed by Daniel Louvard, director of biology at the institute. The novelty of the fixed-term contract arrangement stems from the fact that almost all working scientists in France are civil servants with life tenure. The government is reluctant to create a population of researchers on short-

term contracts. But this has restricted the mobility of young researchers within France and the flow of talent into active research groups.

Louvard says he wants to create a system within France similar to that of the European Molecular Biology Laboratory (EMBL) at Heidelberg, Germany. Louvard himself was trained at EMBL, and later set up a laboratory at the Institut Pasteur in Paris.

Staff at the new institute will be employed on the understanding that they will

IMAGE
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The Institute's buildings in the heart of Paris.

seek positions elsewhere after five years. "We are going to create a breed of scientists [in France] who will know from the beginning that they are going to move," says Louvard. **Declan Butler & Annette Thomas**

Biodiversity treaty misguided

SIR — The 1992 Convention on Biological Diversity addresses a broad spectrum of issues related to the protection of biological diversity. These include the conservation of habitats in developing nations and the availability of resources to make this possible (see D. Putterman *Nature* 371, 553; 1994). This 'Biodiversity Treaty' was conceived as an unprecedented opportunity for industrialized and developing countries to reconcile issues of conservation and access to biological resources. But it is burdened with the problematic question of an international biosafety protocol (regulations) for biotechnology, which in any case has little to do with biodiversity. The kind of biosafety regulation being contemplated — for example, for field trials of improved varieties of potatoes, maize, rice or cassava — would discourage innovative research and development, commit the countries concerned to a poorly conceived highest-common-denominator level of regulation, and deprive domestic regulatory agencies of discretion in setting regulatory policy.

The international bureaucrats discussing the form and implementation of a biosafety protocol reveal their misguided intentions clearly in an official description of the April 1993 proceedings of an expert panel established to implement these aspects of the treaty (Expert Panels Established to Follow-Up on the Convention on Biological Diversity, Report of Panel IV, UNEP/Bio.Div/Panels/Inf. 1, 28 April 1993). According to paragraphs 57 and 58 "a majority of the Panel members believed the organisms covered by a possible protocol should be restricted to genetically modified organisms", along the lines of the European Union's approach, which defines what is regulated not by risk-related criteria, but according to whether molecular techniques of genetic manipulation have been used.

The panel notes that the scope of regulation "does not include organisms modified by traditional breeding methods", regardless of pathogenicity, likelihood of constituting an environmental nuisance, or other potential risks. This approach has been widely discredited, the scientific consensus holding that there is no conceptual difference between organisms crafted with older techniques (such as mutagenesis or hybridization) and the newer molecular techniques (such as gene-splicing), and that regulation should be risk-based. The panel took little notice of a minority viewpoint cited in the report that the scope of regulation proposed was unscientific and "would ignore organisms actually known to present a threat to biodiversity, while focusing on others for which only hypothetical analyses can be

offered". (In support of the minority view, consider that the bacteria that cause anthrax or bubonic plague could be tested — for example, as a pesticide — without regulation, while a field trial of a gene-spliced extended shelf-life tomato would require a government review.)

The wrong-headed regulation in the making would hurt research and development in ways not limited to the developing world, or even to international transfers and transactions. Paragraphs 74 and 75 of the panel report observe ominously that "a majority of the Panel members interpreted the [treaty] language . . . as if both international transfer of organisms and domestic handling and use of organisms were covered. The majority of the Panel members thought that domestic regulation should be covered by a possible protocol."

The primary goals of the treaty are laudable but the mechanisms provided to accomplish them are vague or impotent, while the biosafety protocol is an imminent hazard to the diffusion of biotechnology through the developing world. Its ability to enhance safety in the developing world is extremely doubtful; and the likelihood of its being cost-effective is nil. Moreover, the protocol's possible jurisdiction over strictly domestic activities could effectively take away individual nations' ability to adopt rational policies, thus discouraging innovation. Ironically, the protocol would stifle development of environmentally friendly biotech innovations that can help clean up toxic wastes, purify water and displace agricultural chemicals — products especially needed in the developing world. The treaty is far more likely to cause harm than to make significant inroads towards its goals. It should be abandoned and a new, sound one renegotiated.

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Athletic nonsense

SIR — This has been a remarkable season for athletic track events. Already the 100-metre world record has been broken by 0.01 s while the 10,000-metre world record is now 26 min 52.23 s. Presumably a runner who completes the 10,000 metres in 26 min 52.22 s will be judged to be the new world record holder. I suggest that the measurement of track events to 0.01 seconds is a nonsense and that there is a need for the time measurement to be commensurate with the errors associated with the several variables and assumptions

involved in such measurements which should be familiar to any scientist.

A 10-second 100-metres runner covers 10 cm in 0.01 seconds while a 10,000-metres runner covers just over 6 cm in the same time. The winner is determined by detection of the intersection of a part the body from the torso to neck with the finish line. A wind assistance of less than 2 metres per second is permitted. It is highly unlikely that the length of the 10,000-metre track can be measured with an accuracy to anything like 6 cm. Even thermal expansion of the track will produce comparable errors. For a 25-lap 10,000-metre race, an error of 6 cm corresponds to a systematic error of 2.4 mm in the distance for one lap.

It is assumed that the athletes respond instantaneously to the starter's signal. But within the margin of 0.01 seconds some of the athletes may have anticipated the starter's signal and already covered several centimetres. It is unrealistic to attach any significance to differences in timings of 0.01 seconds. And it is probably unrealistic to quote 100-metre results to better than 0.1 s and for the 10,000-metre race to better than 1 s. In the latter case the uncertainty may even reach a few seconds.

I do not believe that any scientific significance can be attached to the claim that the new 100-metre world record holder ran faster than his predecessor, indeed he may even have been slower.

B. W. Wybourne

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Counting time

SIR — Kukla's communication (*Nature* 372, 124; 1994) is a classic example of the confusion that the BC/AD system of reckoning time generates, even among university professors expert in chronology. The fact is that the time interval between 1.5 years BC and 1.5 years AD (*sic*) is not commensurate with that between the dates 1.5 BC and AD 1.5. Because the years run backward in the BC numeration while time runs forward, and because there is no year zero, there are six months between 00.00 h 1 July, 1 BC (– 1.5 BC) and 00.00 h 1 January, AD 1; and six more months between 00.00 h 1 January and 00.00 h 1 July, AD 1 (– AD 1.5). Therefore, there is only one year between 1.5 BC and AD 1.5, as I said in my original communication. Notice also that the limit of 1.999 . . . BC is AD 1, not 2 BC. The calendar reform I have proposed (*Nature* 366, 716; 1993 and *EOS* 75, 219; 1994) will clear the confusion.

Cesare Emiliani

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Science decided by committee?

SIR — Relativism has at last reached the pages of *Nature*. In her review of a book by K. J. Carpenter on the history of nutrition, Sally M. Horrocks quotes with approval his conclusion that scientific fact is no longer the result of individual endeavour of a single decisive experiment, but of negotiations of committees whose "democratic approach to truth is a contradiction in terms" (*Nature* 372, 329; 1994). I'm sorry to hear that research in nutrition is in such a sorry state. But then Horrocks goes on to say the same is essentially true of all science, as has been demonstrated by the sociologists of science. She apparently supports the relativist view that science is merely a social construct and also believes that "scientific truth has lost its innocence and purity and has become tainted by association with forces outside its control". Such corruption is seen "as a fundamental part of the creation of new scientific facts and ideas".

I am one of those to whom she refers who attack "vehemently" the relativist approach to the sociology of science. Of course science is a social process with many and complex influences. But the validity of scientific ideas has, in the long run, nothing to do with these influences. They stand on their ability to explain nature. My questions, then are these. Does she really believe that the structure of DNA or the genetic code was settled by a committee, corrupt or otherwise? Which papers that *Nature* publishes conform to her relativist stance? Is the whole of modern molecular biology merely a social conspiracy?

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Animal research

SIR — In your description of the future supply of primates for European interests (*Nature* 372, 120; 1994) you wrongly state that the Primate Vaccine Evaluation Network (PVEN) was set up in order to meet the implications of moves by the European Commission (EC)'s environment directorate on the import and use of primates in research.

We write as joint coordinators of PVEN, a joint initiative of the Biomedical Primate Research Centre, Rijswijk, the Fundacion Centro de Primates, Cali, and the EC research directorate. It was founded to help improve the standards and capability for high quality non-human

primate research in source countries, emphasizing research on major health problems of direct relevance to developing countries. The focus is therefore on a particular set of health problems.

Commitment to high standards for animal welfare and stringent criteria for acceptable origins of animals to be used in research are central issues in a set of guidelines shortly to be published by PVEN. Membership of PVEN will entail an accreditation process based upon these guidelines. While cooperation between PVEN, the European Primate Resources Network (EUPREN) and the EC environment directorate, based upon this accreditation, is expected to ensure that primate importation complies with new directives, it is vital to recognize that this is secondary to the main aim of PVEN.

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SIR — Toni Feder¹ quotes Ray Guillery on plans to back animal research through education of "the public, particularly schoolchildren, about the importance and necessity of animal research".

As a scientist, I have myself carried out vivisectionist experimentation using oocyte micro-injection technology to produce transgenic animals^{2,3}. I am currently involved in similar applications on humans, setting up an intracytoplasmic sperm injection (ICSI) facility in a clinical *in vitro* fertilization (IVF) programme. The experience of having injected thousands of mouse eggs turns out to have been invaluable for my work: trying to help almost azoospermic patients become parents by ICSI-IVF.

As a democratic Athenian, I am nevertheless horrified at the idea of any "scientific group" ("research lobby" is the word Feder actually uses), planning to "educate the public, particularly schoolchildren". Considering the overwhelming influence scientists already have in determining how science is taught, I am more concerned that animal rights' groups do not have sufficient opportunity to "educate" schoolchildren. This is not just unfair, it is also not democratic.

Democracy and science are (still) based on plurality and free circulation of information and opinions. All citizens should learn at school how to make objective judgements about social and ethical issues, future scientists in particular. In this (democratic?) world of lobbied, competitive science, one is then forced to ask a

simple question: how can one expect schoolchildren to become scientists if they are to be conditioned by an education which includes indoctrination on the "merits" of animal research?

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Malaria menace

SIR — A recent article in *Nature*¹ comments on gaps in molecular biology in several areas, including malaria. There seems to be some ignorance about the availability of antibiotics in the context of cerebral malaria. Antimalarial drugs are different from antibiotics, which are seldom effective against malaria. So there is a need for the development of more effective antimalarial drugs.

The recent outbreak of cerebral malaria in Rajasthan (India) proved fatal because the affected population had been exposed to the parasite for the first time. The outbreak has been attributed to the construction of a canal which provided mosquito-breeding sites because of water-logging nearby². At the same time, many of those working on the canal came from parts of the country where malaria is endemic, and were thus the source of disease transmission. The onslaught on the immune system of the local population resulted in many deaths.

A great deal of work still needs to be done to study the host and parasite molecules. In this context, we have earlier isolated and sequenced the knob protein gene that induces protuberances on the surface of infected erythrocytes through which they adhere to the cerebral capillaries and are thus partly responsible for cerebral malaria³⁻⁶. Our recent data on the knob protein gene show that there are at least two alleles of this gene in the Indian *Plasmodium falciparum* isolates. One of these alleles is rare whereas the other has a frequency of about 1:8. The major sequence variations occur in the immunogenic domain but there are also certain random point mutations. We still have to establish the correlation of these alleles with the strain of cerebral malaria in Rajasthan.

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Keeping the climate treaty relevant

David G. Victor and Julian E. Salt

Conventional wisdom holds that the proper next step for an effective climate treaty is to negotiate binding targets and timetables for greenhouse gases. But this is not the best approach.

THIS March in Berlin the member countries of the Framework Convention on Climate Change will hold the first meeting of their highest decision-making body — the 'Conference of the Parties'^{1,2}. What should be the way forward for international policy on climate change? Conventional wisdom is that the next step should be a legally binding protocol that sets strict targets and timetables for cutting emissions of the gases that cause greenhouse warming. Many hope that negotiations will begin at the Berlin meeting that will lead to a signed protocol.

But to be effective, such a protocol must be able to change the behaviour of states (and in turn their constituents) so that they protect against harmful climate changes by controlling emissions of greenhouse gases and adapting to changing climates. At present, it will be difficult to support negotiation of a sensible protocol because there is only a thin infrastructure of useful and shared information about what policy changes states might be able to implement. If targets and timetables are negotiated now, the convention risks drifting away from the reality of what can be implemented and what will make it effective.

The next few formative years should be devoted to building a useful information base that focuses on what the parties are actually planning and doing to control global warming. Despite some exceptions³, most other international environmental agreements have failed to gather this information⁴. Even fewer have developed effective mechanisms for reviewing and assessing policies and plans. In this case, things could be different. Most of the key conditions for an effective reporting and review system already exist in nascent form. Targets and timetables should therefore wait, because the contentious, symbolic and noisy nature of such negotiations will distract from the delicate learning and confidence-building spirit that is needed for launching an effective information and review system.

Symbolic targets

The need for tough and legally binding targets is the central policy goal of the greenhouse activist. The convention currently includes a 'soft' target, a loosely worded pledge for developed countries to return emissions of greenhouse gases to 1990 levels by the year 2000 (and to review the adequacy of that commitment at the

meeting in Berlin). Every draft protocol that has been circulated formally among delegates has a legally binding, hard and numerical target as its central element.

Conventional wisdom presumes that targets matter, but there is little supporting evidence that further targets would do much to control emissions at this stage. Even the convention's current target reflects what governments are already doing — not the reverse. Before the convention's language on targets was finalized in 1992, 22 of the 24 members of the OECD (Organization for Economic Cooperation and Development) had already adopted national targets, mostly in symbolic response to domestic environmental pressure and largely independent of the convention. Four had adopted the "Toronto target" (or deeper cuts), named after a 1988 conference in Toronto that made targets popular with a high-profile call for 20% reductions in carbon dioxide emissions by 2005. Since 1991, all European Union (EU) countries have been covered by a pledge to stabilize carbon dioxide emissions at 1990 levels by the year 2000. Targetless under George Bush, the United States is now meeting Bill Clinton's election promise and has pledged to reduce greenhouse emissions to 1990 levels by the year 2000, leaving ambiguous whether emissions would then be stabilized at 1990 levels (see ref. 5 for a review).

Targets have helped focus domestic debates on inexpensive and easy ways to control greenhouse emissions. This has many benefits because reducing carbon dioxide emissions typically reduces other (local and regional) pollutants. Every country that has seriously examined its policy options has concluded, correctly, that some measures exist that will control emissions at zero or negative cost, and that implementing those measures is appropriate. Nearly all OECD countries are implementing some new policies at least partially based on the desire to control emissions of greenhouse gases. Additional targets will not make any difference to this aspect of environmental policy.

Countries and regions have had a tough time meeting (and planning for) their existing greenhouse targets. Developing real policies requires complex and detailed country-level and sectoral studies; policy options and their economic consequences are complex and fundamentally unpredictable. Targets are useful plan-

ning tools; making them legally binding does not help unless countries are willing either to tolerate noncompliance or to overshoot to ensure they are met.

Because targets have large symbolic value and implementing them could be expensive, few countries have a realistic vision of how they will fully implement their targets. Some have developed modest plans that rely on numerous voluntary (but potentially very effective) programmes for emissions control, notably the United States and partially the United Kingdom. The EU's collective target remains a dream because of disputes between members over sharing the burden for controlling greenhouse emissions.

A few countries have implemented more stringent policies, but there is little relationship between their self-declared targets and the policy measures being implemented. Five countries have implemented carbon taxes as part of larger greenhouse policy programmes — roughly in order, from the highest taxers to the lowest: Sweden, Norway, Denmark, Finland and the Netherlands⁵. But the tax rate is unrelated to the specificity or stringency of national targets. The Netherlands has a tougher target than any carbon-taxing country except Denmark, but has implemented the lowest tax rate. Of the four countries that have adopted Toronto-style targets, two (Australia and Austria) have done relatively little while the other two (Denmark and Germany) are controlling emissions more actively. (Nonetheless, all four will probably miss their self-declared targets, although most may meet the softer target in the convention.) Comparisons of the stringency of greenhouse policies across countries is difficult because policies are tailored for each country's situation and implemented with other existing policies such as taxes and subsidies on energy consumption. The essential point is that these targets and policies both reflect domestic pressures — not that targets lead to policies.

In many countries, it may appear that targets are having an effect when actually other stronger factors are at work. The United Kingdom, for example, is undergoing market and labour reforms that are drastically reducing the carbon-intensive coal industry and that, coincidentally, will reduce carbon emissions. Emissions from unified Germany have dropped 14.5% since the collapse of the Eastern economy in 1987. Poor economic performance in

many countries has lowered emissions. Overall the EU is forecast to miss the Union target by perhaps only 3% (ref. 6). The world is generally decarbonizing by switching to cleaner, more efficient gas⁷, and that is probably good for limiting emissions of greenhouse gases (but is unrelated to fears of global warming).

Mark Edwards/Still Pictures

Assessment

More attention should be given to assessing national emissions, policies and plans, rather than whether countries are narrowly 'in compliance' with binding targets. Most international environmental agreements have not led to useful systems, but other experience (in the OECD, the International Monetary Fund, the International Labor Organisation, and GATT) shows how reporting, review and assessment could lead to effective international agreements⁸⁻¹⁴.

To manage climate change policies, three classes of information are needed: accurate, comparable and disaggregated emissions of greenhouse gases; planned policies and measures, with forecasts of their effects on emissions; and standards against which to judge these actions.

The process of compiling emissions inventories is well under way. Since 1991, the Intergovernmental Panel on Climate Change (IPCC) and OECD have rapidly built effective standards for reporting¹⁵. Governments are actively contributing (as is common in reporting systems^{3,4,12}, data reported by national governments are the backbone of IPCC's international system). The EU is also building its own system. Information on policies and measures is similarly moving forward, with the adoption last February of criteria for national reports. Last September, the first reports were due from the parties; at present only 5 of the 23 reports (communications) on emissions, policies and measures required under the convention are delinquent (a relatively good record).

Regarding standards, the convention offers a loose target against which to judge performance. Further, all the developed countries to whom the convention's target rigorously applies have issued their own statements of greenhouse policy goals⁵, which are also useful standards for judging progress. Additional standards are not needed.

The engine that will make these three sources of information a truly useful and shared base of information is periodic and systematic review and assessment. Again, the early signs are very encouraging. Working with the first 15 national reports, a team of experts supplied by governments and intergovernmental organizations and the convention's secretariat has made some broad comparisons and assessments of the adequacy of existing policies and measures¹⁶. Based primarily on countries' own data and forecasts (rather than

Demonstration at the Earth Summit in Rio — what is needed is a way to avoid confrontation so that an effective international agreement can be reached.

independent assessments), the team found that roughly a third will meet their self-declared targets, a third will miss them, and the balance are uncertain. Perhaps half will meet the softer target without further policy measures.

Useful information on national policies and the adequacy of the convention overall will require more in-depth assessment of the national reports. A full system of assessments is scheduled to begin after the Berlin meeting. Most countries now tentatively approve even of the need for 'visits' during that review process, thus allowing a focused dialogue between national authorities and international reviewers (which in other systems has clearly led to more effective reviews and assessments by coupling reports and reviews to the reality of local conditions and by creating pressure on the state to stay in compliance with international treaties^{8,9,12}).

All the submitted national reports and assessments are publicly available, making it likely that nongovernmental pressure groups and other specialist groups will conduct and publicize their own independent assessments. Transparency of the assumptions, modelling methods and data that underlie the governments' national reports remains a serious problem; without transparency it is usually impossible to conduct truly independent assessments and checks on the sensitivity and veracity of government forecasts.

The convention also allows a multilateral consultative process to review questions about implementation. Reminiscent of the dispute panel system of GATT¹¹, the process could be a forum for addressing specific implementation problems — informally shaping interpretation of the agreement — once a detailed framework is in place¹. This system of information and reviews could evolve into a tougher

system for verification of compliance, which will be needed if the convention becomes more stringent in the future and incentives to cheat increase. Most international environmental agreements give inadequate attention to verification^{17,18}.

Roads less travelled

Avoiding targets at this stage could also offer valuable time to consider other means of elaborating the convention. First is joint implementation. Countries could implement some of their emissions control in another country for tradeable credits. Lessons learned from using similar market-based mechanisms to control other pollutants suggest that joint implementation might help lower the costs of abating greenhouse gases. It could also increase the flow of resources from industrialized countries with high emissions to poorer countries who sell or lease their greenhouse abatement potentials, perhaps reducing the intensity of North-South conflicts over funding¹⁹. These avenues are promising, but actually putting joint implementation into operation is complex and contentious: a pilot phase will be discussed at Berlin. The content of any serious protocol on targets and timetables will depend on what is implementable, which in turn depends on the availability of joint implementation to help reduce the costs of implementation — thus waiting for at least some central lessons and political guidance from the pilot phase is crucial.

Second, the parties broadly agree that efforts to abate emissions should include all greenhouse gases, and thus any protocol with a quantified target will probably require legal expression in a form that allows different countries to focus on abating different mixes of greenhouse gases. The principal is elegant and econo-

IMAGE
UNAVAILABLE
FOR COPYRIGHT
REASONS

mic, but in practice multi-gas commitments are difficult to implement. Conventional wisdom holds that the key to multi-gas commitments is adoption of a global warming potential (GWP), which converts emissions of different greenhouse gases into common greenhouse units. Quantifying a GWP is plagued with informational problems and arbitrary choices — the values calculated by IPCC have changed roughly 15% in only 4 years^{20,21}, and uncertainties have increased as researchers learn more about the physical and chemical processes; there is little reason to believe that GWP values are now stable. The fundamental economic basis of the GWP concept and its treatment of comparisons between different time periods has been convincingly attacked²². Options for managing these well-known problems include phasing different gases into the core commitments over time²³, or setting targets in terms of radiative forcing rather than emissions, but none of these ideas has been adequately debated or tested. The consequences are important: most countries whose reports were reviewed by the secretariat last year think their emissions of carbon dioxide will rise, but emissions of other greenhouse gases (primarily methane) are expected to decline¹⁶. The bottom line — compliance with a target that includes all greenhouse gases — is very sensitive to how the component gases are summed.

Third, the persistent problem for the convention is to ensure that negotiations at the international level yield agreements that are implemented. Conventional wisdom is that 'hard law' — a formal protocol to the convention — is best, but supporting evidence for this assertion is thin. Hard law is binding, and countries that take their obligations seriously will force a

tortuous process of legal drafting, ultimately resulting in soft language because the unpredictability of modern economies and policy instruments assures that international climate policies are intrinsically shrouded in doubt about what is implementable. Hard law with soft language may be symbolically valuable, but it will be ineffective. A variety of explicitly softer measures might be better²⁴⁻²⁶ — perhaps a system of detailed standards but codified as 'soft law' and not as a formal treaty. Targets may be useful international planning tools, but as a soft component of larger complex packages²⁷. The idea of a system of loose pledges and then periodic reviews was popular in the course of negotiating the convention but unwisely abandoned when activist countries sought harder targets and timetables.

Fourth, negotiation of the highly symbolic first protocol to the climate convention will automatically entrain other unresolved debates that already haunt these negotiations, particularly the still vague financial commitments to developing countries. To date, virtually all the financial support for developing countries directly connected to the climate convention has come in the form of support for country studies, emissions inventories, and attendance at UN meetings. (There is some very modest funding of greenhouse gas abatement projects via the global environment facility, which has been loosely connected to the convention².) All these uses are highly appropriate at the early stages of the convention. The convention must be careful to sustain developing countries' involvement because virtually every forecast predicts that emissions from those countries will eventually far exceed emissions from the industrialized nations. Global warming is a long-term problem, and ultimately what hap-

pens in the more populous developing world matters most. Developing countries are, broadly, making their participation contingent on a firmer (and larger) financial commitment from the richer countries to pay for greenhouse abatement projects. Why open those issues now, when few donor countries (whose economies are mostly limping out of recession) feel rich enough to offer adequate financial aid to cement a deal that includes financing of emissions controls in the developing world (and when joint implementation is not available to increase the financial flows to developing countries)?

It is not yet clear what should be done to slow global warming, but future steps will be more productive if we first gather and share an abundance of useful information, and build a system that maximizes the opportunities to learn from the variety of policy measures that governments will implement under the convention.

The arguments here lead to five practical lessons for those who want to build an effective convention: (1) don't focus on tough targets and timetables; (2) give the process of gathering and reviewing information the political space to operate; (3) increase the use of national reports (communications), which are the backbone of the system, by urging governments to report fully and in a manner that allows comparisons between countries' data — experience in other contexts shows this is a weak link; (4) support the secretariat that must manage the reporting and review process — we estimate that initially the needs are modest, perhaps a dozen professionals, funded by parties' stable commitments rather than the present system of voluntary trust funds; and (5) design an effective multilateral consultative process so that questions about implementation can be efficiently raised and reviewed. We estimate that two full cycles of reporting and review — 3–6 years at least — will be needed to ensure the foundation is properly in place.

Our strategy also applies to other international issues where substantive negotiations require better appreciation of the reality of states' existing practices. That list includes the convention on biological diversity, which entered into force last year, and the convention to combat desertification, concluded this year. Both need help to find a substantive way forward from existing broad and symbolic agreements. The climate convention can show the way. □

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Making models of symmetry breaking

A strictly symmetrical (if somewhat artificial) dynamical system has been shown to be capable of spontaneous transition between states in which its behaviour is far from symmetrical.

ONE of the most troublesome of all the troublesome concepts with which particle physicists forever assault the rest of us is that of 'spontaneous symmetry breaking'. At its most general, it is the explanation of phenomena that are inherently asymmetrical even when the underlying laws are inherently symmetrical.

In at least the part of the Universe accessible to us, for example, matter consists of electrons with negative charges and of protons with positive charges — neither antimatter, with positively charged electrons and negatively charged antiprotons, nor the mixture of the two kinds of matter that would naively be expected from the apparently undeniable truth that the interactions between leptons and hadrons via the weak and the strong nuclear forces are symmetrical with respect to electric charge. Somewhere, in the formation of our part of the Universe and perhaps the whole of it, there has been spontaneous symmetry breaking.

To remark that a totally symmetrical equal mixture of matter and antimatter would rapidly annihilate itself in present circumstances, and so could not exist, is neither here nor there. The world we live in seems to be one of the two totally asymmetrical dispositions of particles allowed by the underlying laws. The symmetry has been totally broken.

Inevitably, the textbooks are replete with attempts to make the concept more familiar by means of homespun accounts of asymmetries having nothing to do with particle physics. One such is ferromagnetism. Cool a crystal of iron or of magnetite below the Curie temperature in the absence of a magnetic field, and the texture of the crystal will be organized into domains, with patches of differently oriented magnetization whose vector sum is zero. The translational symmetry of the original crystal is broken at the phase transition; the specific free energy of the crystal is least for a patchwork of magnetized domains. Sadly, even this illustration is too abstract; how else, people ask, could it be?

That is why there is more than a little interest in an example now brought to light of a simple model of what may be thought of as a linear conductor of electricity in which spontaneous symmetry breaking appears naturally. The model is the property of M.R. Evans, D.P. Foster, C. Godrèche and D. Mukamel, who between them boast of seven affiliations to four institutions, the Ecole Normale Supérieure in Paris, the Newton Institute at Cambridge, the Saclay complex near Paris and the Weizmann Institute (*Phys.*

Rev. Lett. **74**, 208; 1995). But the model is an intriguing innovation in its own right; its authors' interest is to show that symmetry-breaking can happen in one dimension.

It is built from an array of lattice points along a line, each of which may be occupied by a + charge, a - charge or a hole (labelled 0). If this is to be a model for conduction, there must be microscopic rules for telling how the system changes with time. If + charges move predominantly from left to right (and negative charges the other way), here is how the job may be done. Taking the lattice sites in pairs, in any small interval of time, the configuration +0 turns into 0+, similarly 0- becomes -0 and +- becomes -+. For greater generality, the relative probability of the third process (the physical interchange of opposite charges) may be taken as q , a number either greater or smaller than 1.

To make this a complete model of conduction, there must also be a means of adding + charges to and removing - charges from the left-hand end of the lattice and for adding - charges to and removing + charges from the right-hand end. The rule is that a charge removed from either end is replaced by a hole and that a charge is added only when there is a hole there. In each time interval, the probabilities of addition and removal are α and β respectively. It is crucial that both the dynamics of the system specified by the rules for the evolution of pairs of neighbours and the boundary conditions at the ends are symmetrical with respect to the sign of the charge.

For all those reasons, the natural expectation is that the flow of positive charges (from left to right) and that of negative charges (from right to left) will be equal. There will be two currents, of positive charges in one direction and of negative charges in the other, which will be exactly equal. In no sense will it be possible to say that the current is carried predominantly by one charge or the other. Both contribute equally to the current.

But that, it seems, is not what happens. On the contrary, for appropriate choices of the parameters involved, the positive and negative currents through the lattice may well be unequal. All that has been demonstrated by endless Monte Carlo simulations as well as by a simple probability calculation, which entails supposing that the dynamics of the system can be obtained by applying the rules defining it to quantities that represent the probability that site i , say, on the lattice is occupied by a +, a - or a 0.

Specifically, if these probabilities are $p^+(i)$, $p^-(i)$ and $p^0(i)$ respectively (not the authors' notation), with i running through the integers from 1 to say N , the positive and negative currents J^+ and J^- are written down as $J^+ = p^+(i)(1 - p^+(i+1))$ and its inverse. There are also the boundary conditions to be reckoned with, which boil down to $J^+ = \alpha p^0(1) = \beta p^+(N)$ and the inverse equation for the other end. In this calculation, the authors suppose that the parameter q is simply 1.

The underlying cause of the asymmetry revealed is not self-evident. But the giveaway is the observation that the charge exchange at the two ends of the lattice will be a stochastic process, determined by the rate at which appropriate charges reach them to be removed (with probability β), or by the rate at which holes arrive there to be filled, with probability α . And those rates will be determined not simply by the bulk currents in the lattice, called J^+ and J^- , but by the detailed distribution of the two kinds of charges and of the holes along the lattice as a whole.

In particular, there can arise blockages in the flow of charges along the lattice. If, at some stage, there is a block of holes occupying some central position, and separating - charges to the left from + charges to the right, there will be no chance of it being removed until the charges are removed, one by one, from each end. Similarly, a substantial block of holes near one end of the lattice can have the effect of reducing one of the currents at the expense of the other.

The simplest way of verifying all this is to scribble appropriate diagrams on a piece of paper. But the simulations show the point as clearly as could be expected. The most common asymmetrical condition is one in which the density of carriers of one kind is everywhere high (except near the end of the lattice from which it disappears) and of the other is everywhere low (with the same exception). The current is of course carried by the same carriers. The simulations also show that the system will switch from one asymmetrical state to its inverse at irregular intervals determined by stochastic considerations only, but on a leisurely schedule.

None of this amounts to the clear illustration of spontaneous symmetry breaking for which the world may have been waiting. But the model is that of a dynamical system, if a stochastic one. Its long-term interest no doubt lies in other fields, the modelling of diffusion perhaps, but it may help to illustrate what particle physicists are driving at.

John Maddox

Sponges out of their depth

Michelle Kelly-Borges

SPONGES are impressively adaptable to their environment. But are they biologically plastic to the extent of losing the very characters that define their phylum, the Porifera?

On page 333 of this issue¹, Vacelet and Boury-Esnault describe a deep-sea sponge that possesses an elaborate and atypical mode of feeding, and has lost the characteristic sponge aquiferous system and feeding cells. The organism is a member of the genus *Asbestopluma*, family Cladorhizidae. Hook-shaped spicules are, oddly, located on the outside of body filaments, forming an adhesive surface from which small crustaceans can rarely escape (see cover). Once captive, the prey is gradually enveloped in migrating cells, and digestion is complete after several days. This remarkable sponge is effectively a carnivore.

A twist to the story is that Vacelet and Boury-Esnault found their specimens of *Asbestopluma* in a Mediterranean cave, 18–24 m below the sea surface. The sponge's nearest relatives are always bathyal, bathyo-abyssal and even hadal, one species reaching depths of 8,840 m in the Pacific. Presumably cold water trapped within the cave, together with limited nutrient resources and a lack of light, approximates to the conditions of the deepest Mediterranean². The discovery of these sponges in such a shallow and easily accessible habitat will greatly advance studies of their physiology and life history that are impossible to carry out in their normal deep-sea habitat.

Discovery of *Asbestopluma* within this 'bathyal' cave raises intriguing questions about the origin of this sponge population, as the genus has never previously been found in the Mediterranean. How and when, for instance, did the species arrive in the cave? One possibility is that it originated from a chance colonization event. This new *Asbestopluma* is very similar to a near relative found in the deep North Sea, suggesting that this might be the source of the potential founder. If so, however, the speciation event must have occurred within the past 7,000 years, since submergence of the cave in the Holocene sea-rise, an improbably short time for the evolution of a new species through isolation. Perhaps a more plausible explanation is that the cave inhabitants arrived on strong upwelling currents, from unknown

populations in the deeper, unexplored cliffs, caves and overhangs of the Cassidaigne Canyon, 7 km distant.

However the sponges arrived in the cave, there is also the question of larval supply and population maintenance. We know that 'normal' deep-sea cladorhizid sponges incubate very large embryos, but larvae have never been seen. Vacelet and Boury-Esnault have found only a few small embryos in specimens examined from the cave population, which is unex-



Inside the 'bathyal' cave — doing deep-sea biology at 20 metres.

pected as spermatogenesis is so frequent. This is very different from another sponge in the cave, the hexactinellid *Oopsacas*, which produces embryos and larvae year-round^{2,3}. It is yet to be seen, then, whether the *Asbestopluma* population is capable of a complete sexual reproductive cycle under cave conditions.

The sponge has an unusual hydroid-like morphology. Although the filaments of *Asbestopluma* differ cytologically from the asexual reproductive filaments of other sponge genera, like them they may break or pinch off and re-establish. Asexual reproduction is surprisingly common in a diversity of sponge morphologies, and the recruits generated may have a remarkable behavioural and survivorship capability⁴.

The discovery of macrophagy in *Asbestopluma* leads Vacelet and Boury-Esnault to question the validity of the diagnostic characters that define lower invertebrate phyla. There is the idea that, if *Asbestopluma* were not so clearly related to other Porifera, their unique body plan would qualify them as a distinct phylum. Indeed, there are those who consider that the syncytial organization of glass sponges challenges the retention of the Class Hexactinellida within the Porifera⁵.

There is, however, evidence that most

sponges in the family Cladorhizidae possess hooked filaments, lack an aquiferous system, and are macrophagous. Other filter-feeding invertebrates such as tunicates and bivalves are also macrophagous in deep-sea environments, and up to 30 per cent of the bivalve fauna of deep seas may be macrophagous⁶. It just so happens that a sponge's filter-feeding capacity, conferred by the presence of the diagnostic choanocyte feeding cells, is the very capacity that might be lost in any filter feeder within the deep-sea environment.

As a whole, the sponges form a significant component of worldwide marine benthic communities⁵ and are among the most genetically variable organisms⁷.

They are one of the oldest metazoan groups, and as such studies on sponges have contributed a great deal to our understanding of life on Earth. Their complex immune and cell-aggregation systems have led us to a greater understanding of the role of the cell surface in cell and tissue recognition. Higher metazoan gene homologues in sponges, including the homeobox⁸, ribosomal RNA⁹, lectins, ubiquitin and immunoglobulin-like molecules¹⁰, are being sequenced at an ever-increasing rate. These so-called primitive organisms also possess

the widest diversity of biologically active compounds of any marine phylum.

Apart from its intrinsic interest to those who study the Porifera, then, the paper by Vacelet and Boury-Esnault reaffirms a basic message for biologists in general — it seems that whenever sponges need to do something, they are able to do it. □

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The hot, dry mountains of Venus

Sean Willett

WHEN early exploration of Venus established that the CO₂-rich atmosphere of our nearest planetary neighbour produces greenhouse conditions with surface temperatures averaging around 470 °C, hope of Earth-like surface conditions on Venus vanished. But geophysicists continue to speculate that the variable topography of Venus, as mapped by the Pioneer mission and, more recently, the Magellan mission, represents Earth-like tectonic conditions within the planetary interior. Venus' distinctive highlands, volcanic edifices and steep-sided plateaus suggest a dynamic, evolving planetary surface. Although a debate has developed¹ over whether existing topography is young and active or is simply a relic from tectonic activity that ceased half a billion years ago (see last month's News and Views by J. W. Head²), the mountainous topography of Venus provides valuable information about the properties and processes of the planetary interior, as a paper by Williams *et al.* demonstrates³. By modelling topographic profiles of plateaus on Venus and contrasting the results with similar features on Earth, Williams *et al.* are able to show how the temperature and, possibly more important, the lack of water on Venus affect the formation of planetary mountain ranges.

Williams *et al.* take advantage of the high-quality topographic data obtained from radar altimetry and synthetic aper-

planetary crust directly reflect the properties of the deforming material in the planetary interior. During contraction and uplift, the flanks of mountain ranges attain a critical slope that reflects a balance between tectonic and gravitational forces modulated by the strength of the rock⁴. Changes in slope can therefore be interpreted in terms of changes in rock strength. For example, the existence of a high-elevation, low-relief plateau is often attributed to a decrease in the strength of

appears to be extremely narrow, reflecting the higher temperatures of the venusian crust, and, more important, the lack of water.

The long narrow taper of terrestrial fold-and-thrust belts is consistent with the plastic strength of near-surface rocks only if pore fluid pressures are very high⁶, thereby reducing the effective strength of the rock. In contrast to the Earth, Venus is remarkably dry. Although various theories for the lack of water have been proposed, it appears that the greenhouse conditions that are responsible for the high surface temperatures have effectively desiccated the venusian atmosphere⁷. This lack of water is likely to extend into

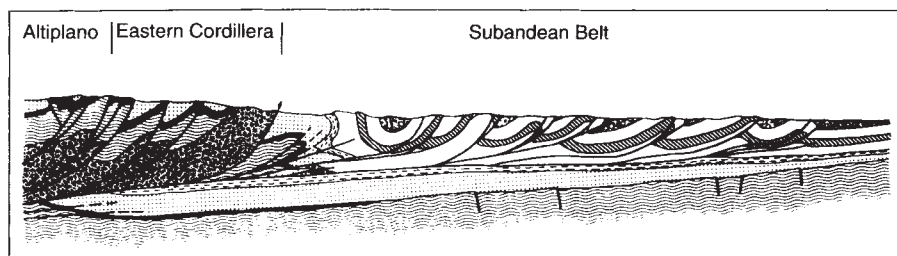


FIG. 2 Geological cross-section from the Altiplano across the eastern margin of the Andes into the Argentina plains, from ref. 3. The Subandean Belt shows the characteristic narrow taper of a terrestrial contractional fold-and-thrust belt. Note the contrast with the steep slopes of Uorsar Rupes shown in Fig. 1.

the lower crust in response to crustal thickening and heating. On Earth, the Tibetan Plateau and the Altiplano of South America (Fig. 2) are examples of high-elevation, low-relief plateaus in the interior of contractional mountain belts. Analogous features on Venus include Maxwell Montes and Uorsar Rupes^{3,5}.

In addition to the intrinsic properties of

both the shallow subsurface, given the lack of meteoric water recharge, and the deep subsurface, as water lost from the venusian mantle to the atmosphere by volcanism is not replaced by subduction of water-rich sediments as occurs on the Earth. The lack of pore fluids is thus the likely explanation for the absence of large, narrow-taper fold-and-thrust belts associated with mountain ranges on Venus.

Another interesting aspect of venusian tectonics is the surprising strength of the lower crust and upper mantle. The very existence of steep-sided, high-elevation plateaus over long timescales attests to the high strength of the venusian lithosphere. Comparison of the topography with the gravity field of Venus confirms that high topographic features are supported by a strong lithosphere⁸. On Earth, at the temperatures typical for the Venus crust, rocks would easily flow, relaxing stresses and topographic features over timescales much shorter than the inferred age of venusian tectonic features. ▶

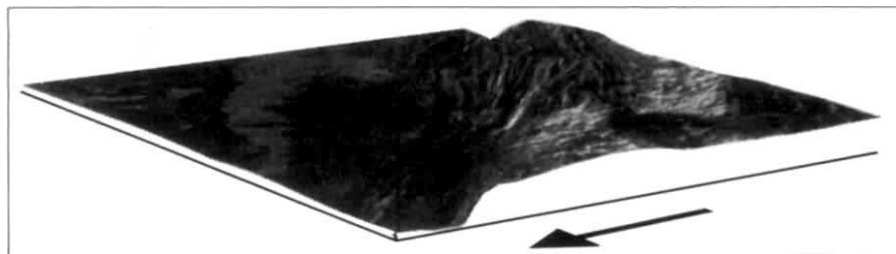


FIG. 1 Synthetic aperture radar image overlaid on altimetry data of Uorsar Rupes on Venus, from ref. 3. The image shows the distinctive high plateau with steep, abrupt bounding slopes. The arrow indicates North.

ture radar (SAR) imagery obtained through the Magellan mission. These data provide nearly global coverage of Venus with a resolution that permits detailed studies of the deformational processes currently or formerly active on Venus. Combining the altimetry and SAR data (Fig. 1) provides a clear picture of topography and morphology of the mountains and plateaus of Venus and provides important calibration data for modelling of the mountain-building processes.

In the modelling framework that they adopt, the topographic profiles of mountain ranges formed by contraction of the

the rock, strength depends on state variables, primarily temperature and pore fluid pressure. It is in these parameters that the differences between venusian and terrestrial tectonics emerge. Large-scale mountain belts on the Earth are typically flanked by broad fold-and-thrust belts where the uppermost crust is contracted into a narrow-taper wedge with a shallow topographic slope extending from the mountain belt interior to the surrounding low-elevation plains. The Subandean Belt east of the Andean Cordillera and Altiplano (Fig. 2) is an example of this structure. On Venus, this structural zone

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Once again, water, or the lack thereof, is thought to be responsible for this planetary contrast in tectonic strength. Recent high-temperature laboratory experiments on the creep strength of rocks that have been thoroughly dried demonstrate that the presence of even a small amount of water profoundly decreases the strength of crustal rocks⁹. Only as the experimentalists have begun simulating conditions on Venus have rock samples been

dried thoroughly enough to detect this sensitivity to small amounts of water. It appears that the very hot, dry conditions of Venus are essential in preserving the strength required to build and support her high-relief mountains. □

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COSMIC RAYS

Accounting for light-element abundances

A. G. W. Cameron

LAST year's discovery¹ of gamma-rays from the Orion star-forming region that were attributable to de-excitation of carbon and oxygen has far-reaching consequences, as it implies an unexpectedly high flux of low-energy cosmic rays in the region. Astrophysicists initially welcomed this as a possible explanation for the high abundances of radioactive nuclides in the interstellar medium^{2,3}, but on page 318 of this issue, Cassé, Lehoucq and Vangioni-Flam⁴ suggest that it could account for the cosmic abundances of the light elements lithium, beryllium and boron.

Because the observed γ -ray lines are broad, it is evident that the γ -rays are emitted from energetic particles, thus indicating a flux of relatively low-energy cosmic rays some 30 times that expected near the Sun (the particles are not detected from the Earth, so they must lie below the low-energy cut-off). At least in the larger star-forming regions, including Orion, supernova explosions will be produced in the more massive stars, and these are natural sources of this extra component of lower-energy cosmic rays. So the excess cosmic rays will be concentrated within such star-forming regions, as it will take some time for them to diffuse out of the local complex tangle of magnetic fields.

Clayton² immediately seized upon this excess flux to suggest that the large amount of the radioactive isotope ^{26}Al in the interstellar medium could have been produced in star-forming regions in this way, and that this and other extinct radioactivities in the early Solar System might have been produced by energetic particle bombardment in the fragment of an interstellar molecular cloud that collapsed to form the Sun. He assumed that a solar-like abundance spectrum of particles had been accelerated in Orion. Such a spectrum, though, would have produced detectable γ -rays in the 1- to 3-MeV region, and these were not seen. Clayton

has therefore retracted his suggestion about the production of ^{26}Al in the interstellar medium but stands by a modified form of his suggestion concerning the extinct radioactivities in the early Solar System. In my view some such bombardment process seems necessary to explain the presence in the early Solar System of the short-lived nuclide ^{41}Ca (ref. 3).

Now Cassé, Lehoucq and Vangioni-Flam⁴ have applied our new-found knowledge about the Orion region to the question of the abundances of Li, Be and B. They start with a large helping of common sense. Because it is the massive stars in the Orion OB1 association that have produced many supernovae and a large interstellar superbubble extending from Orion to Eridanus⁵, then the energetic particles that were accelerated should reflect the composition of the massive envelopes of these stars before their supernova stage. In the pre-supernova evolution and in the explosions themselves, large amounts of helium, carbon and oxygen were synthesized, and therefore it is a reasonable expectation that these elements should dominate in the compositions of the excess low-energy cosmic rays that were accelerated in the aftermath of the explosions. In a symposium last year on the evolution of star-forming regions*, R. Ramaty emphasized that the observed spectrum of Orion γ -rays required carbon and oxygen acceleration but, to minimize the production of 1- to 3-MeV γ -rays, relatively little acceleration of still heavier elements and of hydrogen.

It has long been recognized that Li, Be and B are not products of stellar nucleosynthesis in the same way that nearly all the other elements are, and so the standard explanation for their presence in nature has been to attribute their production to cosmic-ray spallation — the shattering of nuclei through collisions — in the

interstellar medium (with an excess amount of ^7Li attributed to primordial nucleosynthesis). However, if one uses a cosmic-ray energy spectrum given by an inverse power law and known spallation cross-sections with known interstellar medium abundances, straightforward calculations fail to reproduce the relative isotope abundances of these light elements. In general, there is a problem with boron: the B/Be ratio is roughly double the current ratio for stellar sources formed in the early galaxy, and the current $^{11}\text{B}/^{10}\text{B}$ ratio is nearly twice as great as the calculation predicts⁶.

It was recognized⁶ in 1971 that these ratios could be accounted for if an extra source of low-energy cosmic rays could exist above that predicted by the above power-law spectrum, for these would not be directly observable from the Earth. In that case there would be an excess production of ^{11}B relative to ^{10}B owing to the difference in the energy thresholds for the production. Cassé *et al.* have now shown that the excess flux of low-energy cosmic rays in Orion provides a natural explanation for these long-standing discrepancies, and that a plausible galactic evolution calculation can fit the abundances nicely.

There is an interesting footnote to this story. The possibility that the energetic particles in Orion-like regions could produce the missing boron has also been considered by Fields *et al.*⁷, who tentatively reject this idea because they expect that the accelerated particles would produce too many 1–3-MeV γ -rays and that a universal low-energy flux would produce too much ionization of the interstellar medium. They suggest instead that the additional ^{11}B might be produced by neutrino-induced reactions in supernova explosions. Cassé *et al.* avoid these difficulties by proposing that the Orion flux has a composition similar to the envelope abundances produced in a massive supernova explosion, and that the flux is concentrated in star-forming molecular clouds and does not characterize the flux in the general interstellar medium. Many aspects of the Orion puzzle remain unclear, and I expect that there will be more theoretical surprises before the dust settles. □

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A butterfly flutters by

David Baltimore and Amer A. Beg

THE miracle of X-ray crystallography has brought forth from the chrysalis of NF- κ B a butterfly that grips DNA by enveloping it. Two laboratories have solved the biggest DNA-binding domain yet to yield to the combined power of modern X-ray sources, detectors and computers, that of a dimer of the p50 subunit of NF- κ B.

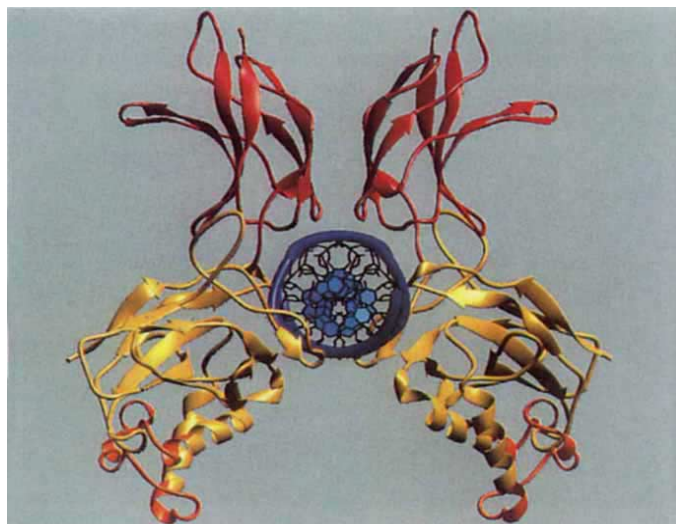
As reported by Ghosh *et al.*¹ and Müller *et al.*² on pages 303 and 311 of this issue, the characteristic Rel-homology region (RHR) in the p50 subunit of the NF- κ B transcription factor consists of two, flexibly linked, domains, both of which bear an uncanny resemblance to an immunoglobulin domain. In its dimeric form, bound to DNA, this RHR has the appearance of a butterfly. Although only the carboxy-terminal domain contributes to the dimer interface, both domains are involved in important DNA interactions. Making about 38 individual contacts with target DNA, the p50 dimer wraps itself around about two-thirds of the cylindrical surface of the double helix, leaving only the DNA's minor groove free.

The NF- κ B transcription factor is a key element of the rapid-response system that allows cells quickly to adjust their transcriptional programme to new challenges. It was discovered and named nine years ago as a nuclear factor (protein) able to bind to the immunoglobulin κ light-chain transcriptional enhancer which appeared to be active only in B lymphocytes³. NF- κ B consists of a dimer of p50 and p65 subunits, both of which have roughly 300-amino-acid regions with clear homology to the Rel proto-oncogene. The genes for each subunit have close relatives, making a family of five vertebrate proteins⁴ and two insect proteins⁵. RHR proteins bind to DNA as either homodimers or heterodimers of various combinations because the RHR has both dimerization and DNA-binding ability.

Very many genes have regulatory regions bearing NF- κ B binding sites (termed κ B sites)⁶. In vertebrates, these genes are primarily involved in processes that respond to external challenges: immune functions, inflammation and the acute-phase response. A unique feature of NF- κ B is its regulation through association with I- κ B proteins. An NF- κ B/I- κ B complex is largely cytoplasmic and unable to bind to DNA. Functional activation of NF- κ B involves dissociation from and

degradation of I- κ B, which frees NF- κ B to translocate to the nucleus and bind to κ B sites in target genes, activating their transcription. Various combinations of RHR proteins as well as NF- κ B are regulated in this fashion.

The structure of p50 bound to DNA is unrelated to that of previously identified DNA-binding proteins. As opposed to the usual DNA recognition by short α -helices, p50 interacts with DNA through loops. Loops on both the carboxy-terminal



Wings that grip — DNA in the embrace of the NF- κ B p50 dimer.

dimerization domain and the amino-terminal domain make phosphate contacts that must make major contributions to the binding energy. One of these loops in the amino-terminal domain of p50 reaches into the DNA and contacts the bases, prompting Müller *et al.* to name it the recognition loop. The proximity of DNA-binding residues to the dimer interface makes for a remarkably seamless set of protein-protein and protein-DNA contacts. NF- κ B binds to DNA with picomolar affinity⁷ compared to the nanomolar affinity of most other DNA-binding proteins. The wrapping of the DNA by NF- κ B and the large number of contacts with DNA accounts for the extraordinary affinity.

Conservation in the recognition loop sequence among RHR proteins explains their ability to bind similar sequences. On the other hand, use of a relatively flexible hinge and limited sequence diversity among the DNA-binding amino acids probably explains their ability to interact with variable affinity with different but related sites. It is indicative of the flexibility of the RHR interaction with DNA that the two structures^{1,2}, although quite similar, have a fundamentally different rela-

tionship to the chosen target DNAs. It is noteworthy that the minor groove of DNA is exposed in these structures, which explains the ability of minor-groove-binding proteins such as the high mobility group protein I(Y) — HMGI(Y) — to interact with DNA along with NF- κ B (ref. 8).

Dimerization comes about through a conserved core of interdigitating hydrophobic side-chains and surrounding polar contacts. A natural, dimerization-deficient, alternatively spliced form of p65 (p65 Δ) has been identified which lacks amino acids 222 to 231 of wild-type p65 (ref. 9). Although not part of the dimer interface, the related residues in p50 link

two regions which form the interface.

Cytoplasmic retention of RHR proteins through I- κ B binding is thought to occur at least partially by direct masking of nuclear localization signals¹⁰. Several members of the I- κ B family have been identified thus far, all of which have 33-amino-acid ankyrin repeats which are necessary for interaction with the RHR. Mutagenesis within the RHR suggests that the I- κ B binding site is at least partially in the cleft formed by the dimerization domains of the two p50 monomers. The nuclear localization signal is not structured well enough to be visible but is likely to reside near the top lips of this cleft. I- κ B proteins not only inhibit DNA binding of NF- κ B but even induce its dissociation when bound¹¹. Perhaps interaction with I- κ B causes a conformational change in the RHR, inducing dissociation from DNA; alternatively, I- κ B could reach down to the DNA interface. The flexibility of the interdomain link also suggests that, once having induced dissociation, the I- κ B might bind to the amino-terminal domain, locking the structure.

Understanding a structure is a first step towards a deeper understanding of function and eventually towards rational

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intervention. Among the functional issues that are not yet illuminated are the specificity of particular RHR combinations towards particular κ B sites and the ability of RHR proteins to activate transcription. Moreover, it is unclear how RHR proteins can interact with many other transcription factors.

Finally, the evolutionary significance

of the resemblance to immunoglobulins, although thought-provoking, is still unclear. NF- κ B and its relatives are involved in important cellular responses that protect animals from various pathogenic challenges. Our recent knock-out of the p50 gene produces serious defects in both specific and nonspecific immune responses¹², showing clearly the import-

ance of this protein. Perhaps the structures will provide the clue for designing inhibitors or activators with therapeutic value. □

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OBITUARY

Eugene Wigner (1902–1995)

EUGENE Wigner died in Princeton, New Jersey on 1 January 1995. In his passing we have lost one of the last of the giants who contributed so brilliantly to the evolution of the field of wave mechanics starting in the 1920s.

He was born in 1902 into the stimulating Jewish community of Budapest. Under the influence of his beloved teacher, László Rátz, and a boyhood friendship with John von Neumann, he rapidly developed the mathematical talents which were a permanent part of his strength.

In fulfilment of a promise to his practical father, he went to Berlin to study chemistry and chemical engineering, of which he soon became a master. However, his heart and all his spare time were devoted to the activities of the brilliant group of theoretical physicists then in or near Berlin. At that time, several other Hungarians such as Michael Polanyi and Leo Szilard, as well as von Neumann, were also in Berlin and formed close bonds. Polanyi, in fact, arranged for Wigner to receive an appointment as assistant to the theoretical physicist Richard Becker, which was then followed by an appointment in Göttingen where, to Wigner's joy, he and von Neumann were reunited. It was during this period that Wigner introduced the enormously powerful methods of group theory into physics.

Von Neumann foresaw, even in the mid-1920s, that there would probably be another great war and it would be coupled to the rising anti-semitism that he was witnessing in Germany. So when in 1929 Princeton University offered him a professorship with tenure he was delighted to establish partly American roots. As he wished, however, to retain links with Göttingen, he suggested that he and Wigner share the appointment. Princeton agreed, being anxious to capture von Neumann on any basis. Thus in 1930 Wigner began his lifelong career in the United States. I became his first American graduate student in 1932 and thus formed a long-standing relationship.

Von Neumann transplanted easily; Wigner did not. He was a lonely man in Princeton for the first few years. Hitler, however, cancelled his German appointment in 1933 and Wigner began to establish roots in what became his most beloved home. The 1930s represented intensely productive years for Wigner, during which he made weighty contributions to solid-state physics, nuclear



Eugene Wigner (at blackboard) discussing physics with fellow Hungarian Edward Teller. From *The Recollections of Eugene P. Wigner* by Andrew Szanton (Plenum, New York, 1992).

theory and the formulation of the group representations associated with the Lorentz transformation. His pioneering work in nuclear physics, based partly on the use of group theory, was to earn him a Nobel Prize in 1963.

As the Second World War approached, Wigner became increasingly apprehensive about the turn of international affairs. When the discovery of nuclear fission was announced early in 1939, he was prepared to collaborate with Szilard and Enrico Fermi who were now at Columbia University to see if it would be possible to create a nuclear chain reaction. The rest is history. Once it was clear that at least two neutrons were produced during fission, that plutonium was doubtless fissionable and that pure graphite could serve as a moderator, all of Wigner's talents, active and latent, were unleashed. In 1942 he and many colleagues settled at the University of Chicago where more space for the work was available, to continue their work with renewed activity under the leadership of Arthur Compton.

While Fermi was beginning construc-

tion of the first experimental working reactor, which went critical in December 1942, Wigner, with a small group of highly talented individuals that included Alvin M. Weinberg, Gale Young and Edward C. Creutz, was laying out detailed engineering plans for the construction of the gigantic nuclear reactors that were ultimately built at Hanford. This work went hand-in-hand with much experimental work of a technical nature.

Wigner's training as a chemical engineer was finally paying off on a big scale. He suffered some consternation when General Leslie Groves turned control of the construction of the reactors over to the Dupont Company. Wigner feared that the delays that would inevitably be involved in such a transition could prove fatal if it came to a close race with the Germans in taking advantage of nuclear fission. Nevertheless, he continued to play a magnificently responsible role in co-

operation with the Dupont team.

Wigner so abhorred the thought that the first full-scale use of nuclear fission would be to destroy human lives that he signed the 1945 petitions protesting against any use of nuclear bombs in Japan. He, and many of his team, including Weinberg and Young, began in 1946 to develop plans for nuclear reactors (including breeders) for peaceful purposes. Once it became clear, however, that the field would become embroiled in complex political matters, he returned to academic life in 1947. There he focused once again on theoretical and nuclear physics but continued to advise on problems dealing with nuclear reactors. Anticipating that there would eventually be a widespread distribution of nuclear weapons, he spent much spare time at Oak Ridge working on practical plans for providing civil defence. It was part of his sense of obligation to fellow citizens and, indeed, all humanity. Frederick Seitz

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Neuron saving schemes

Ronald M. Lindsay

THE discovery that neuronal loss can be alleviated by neurotrophic factors, such as nerve growth factor, has generated a great deal of interest in the therapeutic potential of these molecules¹⁻³. Degeneration of motor neurons in amyotrophic lateral sclerosis (ALS) and of dopamine neurons in Parkinson's disease has been the subject of particular attention in this respect^{4,5}, and data reported in five papers now open up fresh prospects for research. Four of the papers — by Tomac *et al.*⁶, Beck *et al.*⁷, Yan *et al.*⁸ and Oppenheim *et al.*⁹ — appear elsewhere in this issue; the other, by Henderson *et al.*¹⁰, was published late last year in *Science*. Taken together, they indicate that glial-cell-line-derived neurotrophic factor (GDNF) may repay further evaluation for treating neuron loss in both ALS and Parkinson's disease.

The application of molecular techniques to neurobiology has brought about a huge increase in the number of fully characterized neurotrophic factors that promote neuronal survival. In the process, the classical definition of a neurotrophic factor — that of a neuronal-specific, survival-promoting molecule found in limited amounts in the target-fields of responsive neurons — has had to be re-evaluated. This is partly because the prototypical neurotrophic factor, nerve growth factor (NGF), has actions on non-neuronal cells and its synthesis is not restricted to target cells; partly, and more seriously, because it has emerged that certain mitogens and cytokines have

NGF-like actions on neurons (see table); and partly because some neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), seem to have paracrine or autocrine functions.

Perhaps surprisingly, given that it was described only two years ago, GDNF was not identified and characterized by state-of-the-art molecular techniques, but in the good old-fashioned way — as a conditioned-medium activity that was found to support survival and differentiation of cultured dopamine neurons. Purification, sequencing and cloning revealed it to be a distant member of the transforming growth factor- β superfamily¹¹. Faced with the problem of defining a factor on limited data, GDNF was initially taken to be a highly specific neurotrophic factor for dopamine neurons. That it has potent effects on dopamine neurons *in vivo*, as well as in cell culture, finds strong support in the observations of Tomac *et al.*⁶ and Beck *et al.*⁷. But its broader actions are clear from the studies of Yan *et al.*⁸, Oppenheim *et al.*⁹ and Henderson *et al.*¹⁰, all of which show that GDNF has neurotrophic effects on developing motor neurons.

There are various consistencies and discrepancies in the five papers with regard to the neuronal specificity and purported remarkable potency of GDNF. Beck and colleagues⁷ confirm that GDNF is a survival factor for cultured dopamine neurons, whereas Henderson *et al.*¹⁰ report the novel finding that GDNF is a survival factor for purified rat embryo

spinal motor neurons. Although it is claimed to be more potent than BDNF and neurotrophin 4/5 (NT-4/5), GDNF has in fact a similar efficacy. GDNF is expressed in the developing limb bud, suggesting that it has a target-derived role in motor neuron development. Expression in ventral roots may indicate that GDNF supports motor neurons before target encounter.

A limited survey of the influence of GDNF on neurons of the peripheral nervous system indicates that it does not promote survival of trigeminal sensory neurons or sympathetic neurons. The effects of GDNF do, however, seem to overlap with those of both BDNF and ciliary neurotrophic factor (CNTF) in enhancing survival of nodose sensory neurons. Oppenheim *et al.*⁹ report that survival of chick embryo motor neurons in culture is improved by GDNF, with a potency similar to that of CNTF or BDNF. Other data in this paper suggest that GDNF may indeed have effects on sympathetic neurons, as GDNF treatment *in ovo* increases the number of neurons in sympathetic ganglia — implying that these neurons are rescued from programmed cell death. However, GDNF does not reduce programmed cell death in other peripheral ganglia (ciliary, nodose, dorsal root) or in nuclei in the central nervous system, with the exception of motor neurons and interneurons of the isthmus-optic nucleus. Spinal sensory neurons show receptor-mediated retrograde axonal transport of GDNF⁸, suggesting that they respond to the factor.

Three reports⁸⁻¹¹ indicate that GDNF affords almost complete rescue of the >80 per cent loss of motor neurons that follows facial nerve axotomy in the neonatal ro-

Activity of neurotrophic factors

Ligand (receptor)	Motor neurons		Dopamine neurons	
	Survival <i>in vitro</i>	Protect/rescue <i>in vivo</i>	Survival <i>in vitro</i>	Protect/rescue <i>in vivo</i>
GDNF (not known)	++++	Neonatal axotomy: survival, no atrophy Adult axotomy: ChAT phenotype	+++	Adult axotomy: survival, TH phenotype MPTP toxicity: protection and recovery of TH, dopamine metabolites, motor behaviours
CNTF (CNTFR- α , LIFR, gp130)	++(+)	Neonatal axotomy: survival Efficacy in mice with motor neuron disease: <i>pmn</i> , <i>Wobbler</i>	—	Adult axotomy: survival
NGF (TrkA)	—	—	—	—
BDNF (TrkB)	++++	Neonatal axotomy: survival Adult axotomy: ChAT phenotype	+++	Adult axotomy: survival, TH phenotype
NT-4/5 (TrkB)	++++	Adult axotomy: ChAT phenotype	++++	Adult axotomy: survival, TH phenotype
FGF (FGFR)	++(+)		Indirect	MPTP toxicity: survival, TH phenotype dopamine
PDGF (PDGFR)	Not known	Not known	++	Not known
IGF-1	Not known	Terminal sprouting	+	Not known

Data mostly taken from refs 1-3. Efficacy *in vitro* runs from — (no effect) to ++++ (almost complete survival). GDNF, glial-cell-line-derived neurotrophic factor; TH, tyrosine hydroxylase; TGF β , transforming growth factor- β ; CNTF, ciliary neurotrophic factor; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT-4/5, neurotrophin 4/5; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; IGF-1, insulin-like growth factor-1. ChAT phenotype indicates expression of choline acetyltransferase. TH phenotype indicates expression of tyrosine hydroxylase; MPTP, the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

dent. Uniquely, GDNF seems to prevent both loss of neurons and atrophy of the cell body, though taken alone the clinical relevance of these striking results is debatable. Other studies indicate that the previously reported rescue effects of CNTF (ref. 12) or BDNF (ref. 13) in this rodent model are quite transient^{14,15}. Moreover, as a further caution against overinterpretation of animal data, in the *pmn* (progressive motor neuronopathy) mouse mutant, in which CNTF treatment prevents degeneration of motor neurons and thus extends the lifespan of affected animals¹⁶, GDNF rescues motor neurons but has no effect on the premature death of the mice (A. C. Kato and P. Aebischer, personal communication).

Given its initial billing as a highly specific survival and differentiation factor for dopamine neurons, the results of testing GDNF in animal models of Parkinson's disease have been keenly awaited. Tomac *et al.*⁶ and Beck *et al.*⁷ provide substantial evidence that GDNF does indeed protect or restore function of dopamine neurons compromised by axotomy or the neurotoxin MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). Although the clinical relevance of their model is questionable, Beck *et al.*⁷ clearly show that repeated intranigral injections of GDNF largely attenuate the 40 per cent, or more, loss of dopamine neurons resulting from transection of their axons within the median forebrain bundle. Although this demonstrates the responsiveness of mature dopamine neurons to GDNF, such rescue of axotomized cell bodies does not restore behavioural deficits and so provides only indirect evidence of any clinical usefulness.

However, in a more relevant test of the potential of GDNF to protect or restore dopaminergic function, Tomac *et al.*⁶ provide encouraging data from a mouse model of Parkinson's disease produced by the dopaminergic neurotoxin MPTP (which is known to produce parkinsonian symptoms in humans). In one experiment, the authors show that intrastriatal injection of GDNF before administration of MPTP attenuated toxin-induced changes in levels of dopamine and its metabolites, preserved striatal levels of the important enzyme tyrosine hydroxylase, and improved scores in tests of motor function. Furthermore — and this is arguably the most exciting finding — treatment of mice with GDNF days after MPTP treatment produced a substantial regeneration of tyrosine-hydroxylase-immunopositive fibres within the striatum, partially restored dopamine levels and improved motor performance.

In all, these effects of GDNF on dopamine neurons are encouraging. But it should be noted that both groups^{6,7} resorted to direct intraparenchymal injection of GDNF — this procedure has never

been carried out on human subjects, and we don't know about the possible anatomical or behavioural side effects, or how prolonged the restorative effects of GDNF might be.

As the aetiologies of Parkinson's disease and sporadic ALS are unknown, devising ways to arrest these diseases rely largely on broad concepts of how best to interfere with neuronal atrophy and loss. Oxidative stress, excessive Ca^{2+} and glutamate toxicity are high on the list of suspects, but the basic biochemical nature of these processes has made it difficult to prove their guilt or to abolish their deleterious actions on neurons. The striking effects of neurotrophic factors in cell culture and the long list of accomplishments of NGF (and now a variety of other factors) in animal studies makes these molecules attractive candidates as therapeutic agents. But animal models can be poor predictors of adverse reactions in patients. This is highlighted by the confounding side effects of hyperalgesia and weight loss found in clinical trials of NGF (ref. 17) and CNTF (J. M. Cedarbaum, unpublished results), respectively, despite the strong rationale behind these trials and these factors' efficacy in animal studies. Such side effects may, however, be overcome by using combinations of growth factors that have synergetic effects at dose levels that show no side effects¹⁸. Clinical studies with CNTF, BDNF and insulin-like growth factor-1 (IGF-1) in ALS patients are in progress.

The new reports on GDNF biology highlight how quickly the discovery of ligands is followed by assessment of their therapeutic potential. But further animal studies, and more detail on the distribution of GDNF-binding sites and its general pharmacology, pharmacokinetics, stability and toxicology, are necessary before we can see just how promising that potential is. □

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Recycled teeth

LAST week Daedalus was musing on the atomic bomb, in which a hollow shell of high explosive is detonated to compress a central charge of plutonium. He wanted to use the technique on samples or assemblies of animal teeth. Under the sudden massive pressure they should flow plastically, and pressure-weld together into an artificial ivory. He is now applying the idea to human teeth.

Restorative dentistry is bedevilled by the lack of any good substitute for tooth material. Gold is expensive, amalgam slowly erodes, porcelain is hard to shape, and none of them bonds firmly to the tooth beneath. But implosive forming, says Daedalus, would permit human tooth substance itself to be formed into crowns and inserts. The dentist would take the usual wax or elastomer impression, but instead of casting a crown from it, its shape would be scanned into a computer. A program adapted from those of bomb physics would then calculate what pattern of explosive charges would deform what initial sample of dentine into that exact shape. The program could even do the calculation for a composite sample, such as a chunk of dentine in contact with a sliver of tooth enamel. The explosion would pressure-weld the two into a single insert of the desired shape, faced with a hard enamel biting surface.

The ideal material for such an insert would, of course, be tooth substance from the patient himself. This would be immunologically compatible with its new site. It should be welcomed back into the body, and should bind firmly into place by slow protein infiltration. But where to get a sample of the patient's tooth substance, without pulling out another of his teeth? Daedalus recalls the 'milk teeth' we all shed as infants, from the age of about six. These teeth should not be discarded or sold to the tooth fairy, but carefully preserved in liquid nitrogen. They would then be available as raw material for subsequent dental repairs to their original owner.

We grow and shed only 20 milk teeth; they are quite small, and have no root (it is resorbed in the shedding process). This modest reserve of material should still provide a lifetime's supply of crowns and inserts, and could even give a whole replacement tooth as well. To fit in the jaw, such a tooth would need a properly shaped root. It would have to be pressure-welded from several milk teeth, and then forced into its place in the mouth. It should soon bind fast to the compatible tissue all around it, and would then be as good as new. Indeed, it would be even better — having no nerve, it could never ache.

David Jones

Cause of blue petal colour

SIR — The question of how blue flower colour develops is a fascinating problem which has long attracted attention^{1,2}. Early this century Willstätter proposed the pH theory³, and soon after, Shibata and Shibata put forward their metal complex theory⁴ on the basis of the finding that the vacuolar pH (pH_v) in plant cells is generally around 5, and more acidic than that of the cytosol⁵. We were earlier able to confirm the validity of the metal complex

at pH 6.40 did not result in any bathochromic shift, suggesting no participation of metal complexation.

Light microscopy of transverse sections of petals confirmed that HBA was distributed homogeneously in the vacuoles of both types of epidermal cells, but not in the vacuoles of the parenchyma cells. For measuring pH_v a cut petal was set on a Plexiglas vessel with its abaxial side uppermost and the tip of a double-barrelled

pH microelectrode¹¹ was inserted into the epidermal cells. The pH profile gained by gradually advancing the electrode tips exhibited three stable pH stages (Fig. 2). The pH value of the first inserted abaxial epidermal cell of a blue petal was higher than 7.5 and that of its redepidermis was around 6.6. After the first advancement (Fig. 2, first arrow), the pH suddenly decreased to around

6 and with a further advancement (second arrow) increased to 7.7 in blue petals and to 6.9 in the red petal case. The low pH value of the second stage can be assigned to the pH_v of the colourless parenchyma cells and, thus, the electrodes can be concluded to have been consistently inserted into vacuoles (table). When flowers were exposed to, high ambient concentration of CO₂ gas, the petal colour changed to purple immediately. This colour change proved reversible upon return to air. The pH_v of the CO₂-treated cells was found to be 6.9 (see table), and thus the colour

Flower	Cell location	pH_v (no. of experiments)
Blue open flower	Abaxial epidermis	$7.7 \pm 0.2^*, \#$ (26)
Purplish red bud	Abaxial epidermis	$6.6 \pm 0.4^{**}, \#$ (22)
Purple flower (CO ₂ treated)	Abaxial epidermis	$6.9 \pm 0.3^{**}, \#$ (7)
Blue open flower	Parenchyma	$6.0 \pm 0.4 \##$ (16)
Purplish red bud	Parenchyma	$5.8 \pm 0.5 \##$ (7)

Data fluctuated within ± 0.1 pH unit per min. Values are means $\pm$ s. d. (no. of experiments). Significant differences ($P < 0.001$) were obtained between the * and **, and # and ##.

theory for commelinin, the blue pigment of *Commelina communis*, by structural determination⁶. However, pH microspectrophotometric measurements, by Asen *et al.*, of blue morning glory petals indicated an alkaline cell sap, which could be responsible for the blue flower colour⁷. Their methods were not able to assess pH_v directly in living cells and therefore we have measured the pH_v of individual blue and red coloured living cells, of morning glory using a proton selective microelectrode⁸.

The open flower of the morning glory, *Ipomoea tricolor* cv. heavenly blue, is light blue, although the buds are purplish red (Fig. 1a). We determined the structure of heavenly blue anthocyanin (HBA, Fig. 1b)⁹ and confirmed using HPLC that both red buds and blue open flowers contain only HBA as a pigment. In aqueous solutions of various pH the colour of this pigment varied widely with variation in pH¹. The spectra of HBA in aqueous solutions at pH values of 7.68 and 6.37 corresponded to the reflective spectra of the open petal and the bud, respectively, and their colours were stabilized by intramolecular stacking of caffeic acid residues (sandwich-type tacking)^{1,10}. Addition of Al³⁺ or Fe³⁺ ions to the HBA solution

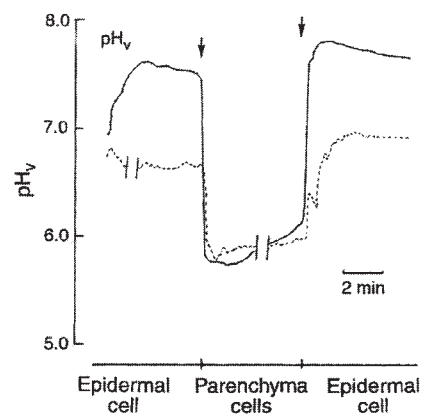


FIG. 2 Profiles of the vacuolar pH (pH_v) of blue open flower-petal (solid line) and red bud-petal (dotted lines) cells using double-barrelled pH-sensitive microelectrodes¹¹. The tip was inserted at an angle of 45° into the cells under microscopic observation. A three-step change in pH values was observed when the tip was advanced stepwise. Arrows, advance of the electrode.

change correlated with the pH_v just as in the *in vitro* case with isolated pigment.

The present study provides clear evidence that colour variation in the blue flower petals of *Ipomoea tricolor* cv. heavenly blue is caused by the unusually high vacuolar pH. Independent of whether metal complexation⁵ or a high vacuolar pH is the causal stimulus, the mechanism of blue-colour development involves an anionic anhydrobase, the quinonoidal anion of anthocyanin².

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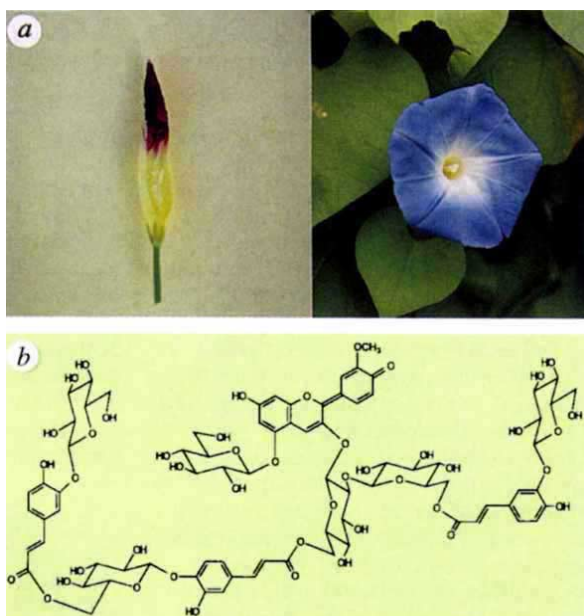


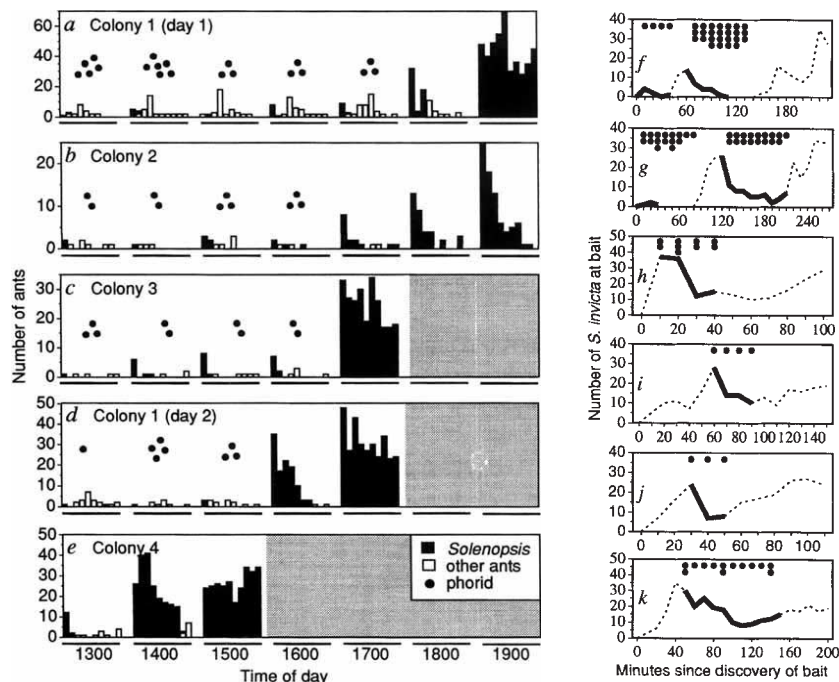
FIG. 1a, Blue blooming flower (right) and the bud (left) of *Ipomoea tricolor* cv. heavenly blue. b, Structure of heavenly blue anthocyanin (HBA)⁹.

Flies suppress fire ants

SIR — Exotic pest species often become dominant in alien ecosystems by escaping regulation by coevolved pathogenic and parasitic organisms¹. Since arriving from its native Brazil around 1940 (ref. 2), the red imported fire ant (*Solenopsis invicta*) has decimated arthropod communities³ and occupied more than 250 million acres in the southern United States². Pesticides have failed to control *S. invicta*, which reinvades poisoned sites more effectively than other ants⁴. Its densities are 5–10 times lower in Brazil than in the United States⁵, yet no natural factor in Brazil has emerged as a strong candidate for biological control⁶. Here we demonstrate that the competitive dominance of *S. invicta* at food resources in Brazil is diminished under attack by flies in the family Phoridae.

Many phorid species are host-specific parasitoids of adult ants⁷. In their presence, host ants attempting to avoid attack lose competitive interactions at food resources⁸ and reduce foraging^{9,10}. *S. invicta* in Brazil is host to many phorid species⁷, all of which are absent in North America. To date no demonstration exists that phorids affect the competitive ability of an ecologically dominant ant species. We examined the influence of parasitoids on *S. invicta*'s competitive success in Brazil by manipulating the presence of phorid flies and observing bait occupancy by *S. invicta* and other ant species (a–e in the figure).

Fire-ant workers recruiting to the baits were attacked by the phorid *Pseudacteon solenopsidis*, which hovered less than a centimetre in front of an ant and maintained its position by flying backwards as the ant fled. To oviposit, the fly turned 180°, darted quickly down and inserted its ovipositor in the foramen behind the ant's head. Ants usually hid under seeds, pebbles and sticks; returned underground; or assumed a defensive posture by lowering their head and raising their gaster. Chases lasted as long as two minutes, with ants fleeing up to a metre from the foraging



Fire ants (*S. invicta*) surged from 4 colonies to displace other ant species from baits independently of time of day and only when phorid parasitoids departed (a–e). Ten baits, each consisting of a 0.5-cm³ piece of cow heart muscle staked to the ground with an insect pin, were placed at 10-cm intervals away from each colony. All ants standing on or feeding at each bait were counted every hour until fire ants dominated all baits. Colony 1 was used twice: on day 1 (a) phorids departed naturally at dusk, and on day 2 (d) phorids were experimentally removed 3 h before dusk. Filled bars, *S. invicta*; open bars, species of the genera *Camponotus*, *Pheidole*, *Conomyrma* and *Ectatomma*. Bars farther to the left at each hour represent baits closer to the mound. Solid circles represent maximum number of phorids attacking *S. invicta* during the 30-min interval before each ant count. Dominance of fire ants in stippled areas of each panel can be assumed because fire ants were never displaced from baits by other ant species in the absence of phorid parasitoids during 6 weeks of field observations. No other ant species visiting the baits was attacked by phorid parasitoids. Numbers of other ants may be a more conservative estimate of their importance than biomass, since a single large *Ectatomma* could steal an entire food resource. Panels f, g, show 10-min observations at the bait closest to the mound in d and c, respectively. Colonies in a–g were on the periphery road of the Mata Santa Genebra Reserve, near Campinas, São Paulo state. Panels h–k show 4 different fire ant mounds outside Dourados, Brazil attacked by the phorid species *P. nocens*, *P. curvatus* and *P. litoralis*, where a single bait consisting of tuna packed into a 3.5-cm straw was placed 15 cm from a colony. Lines show numbers of *S. invicta* standing on or feeding at the bait when phorids were absent (dotted line) or present (solid).

trail. A lone phorid (d in the figure; 1300 h) encountered a fire ant and elicited a defensive response on 30 occasions in 8 min. During this time, fewer than 20 *S. invicta* entered a 5-cm-wide trail between the mound and the first bait; hence, a single phorid was sufficient to disrupt recruitment

Twice during these experiments brief natural absences of all phorid parasitoids occurred in the area around the mound. On both occasions, *S. invicta* recruited rapidly to baits and then retreated when phorids returned (f, g in the figure). This striking pattern of recruitment in the absence of phorid parasitoids and retreat on their arrival occurred in response to three other phorid species at a separate location, Dourados, Mato Grosso do Sul (h–k). Thus, of the seven colonies attacked by phorids during these experiments, fire ant abundance at baits was

always greater in the absence than in the presence of phorids ($P < 0.008$, 1-tailed sign test).

The behavioural distractions caused by phorids reduce the competitive dominance of *S. invicta* in Brazil and might also diminish its ability to dominate in its introduced range. Due to their extreme host specificity⁷, phorids are a good candidate for biological control¹, although their affinity for North American ants must first be understood. Introduction of *S. invicta* specific phorid flies into the United States

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references.

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might contribute not only to fire ant control but also to an understanding of parasitism's role in structuring natural communities.

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Leukaemia and wartime evacuees

SIR — A high degree of mixing of urban and rural groups of people is associated with significant increases of childhood leukaemia, a phenomenon that is consistent with an infective process being responsible¹⁻⁷.

The evacuation by the government of large numbers of urban children during the Second World War is such a situation, which we have recently studied in relation to childhood leukaemia⁸.

The 476 rural districts of England and Wales were grouped into three categories with similar numbers of local children but with contrasting proportions of government evacuees in 1941. In the 'high' category, leukaemia mortality in the years 1945-49 (the earliest period for which data are available) showed a 47% excess relative to the 'low' category, with a significant trend across the categories. Doubt has been cast in Scientific Correspondence⁹ on this infection-based hypothesis because mortality rates from childhood leukaemia in England and Wales showed no sudden increase during or just after the war. National mortality rates are inevitably rather insensitive to changes in rural areas, given the small proportion of the country's children who live there (and in fact the 'high' rural category held only 6.3%). Wartime evacuation therefore does not represent an exception in this context.

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Organic carbon in sediments

SIR — Hartgers *et al.*¹ convincingly demonstrate the presence of derivatives of isorenieratene and a novel C₄₀ diaromatic carotenoid in the aromatic hydrocarbon fractions of Duvernay Formation sourced crude oils, and in chemical and pyrolytic degradation products of associated crude oil asphaltene and source rock kerogens. From this they argue that there are contributions from green sulphur bacterial components in the sedimentary organic matter in the Palaeozoic sediments of Western Canada and elsewhere. The authors further suggest that because these biomarker compounds — indicative of a specific group of photosynthetic bacteria — show carbon isotope signatures significantly different from the bulk total organic carbon (TOC), these organisms have not contributed significantly to the carbon preserved in the sediments from Western Canada and the Williston basins.

We agree with this conclusion, as well as with the argument of Hartgers *et al.*¹ that substantial biases occur in the quantitative interpretation of biological marker data in terms of source biomass contribution to bulk TOC. This bias is likely to occur in quantitative assessments of biological marker contributions from bound fractions, particularly when relative concentrations of components in gas chromatograms are used.

Nevertheless, we are cautious about the overall general conclusion of the paper¹. The inference that bacterial biomass (implying all bacteria) is a minor component of sedimentary organic matter has not yet been tested and, indeed, remains one of the most important questions in biogeochemistry. Hartgers *et al.*¹ suggest that selective preservation of specific biomarker compounds has led to overstating their significance as indicators of sources of sedimentary organic matter. Although undoubtedly true in this specific case, and perhaps more widely², this suggestion does obscure the wider, more significant, issue that microorganisms other than phototautotrophs are likely to contribute considerable amounts of organic carbon to sedimentary basins. The arguments for this are twofold: (1) the work of Parkes *et al.*³ shows that there is significant bacterial production resulting from growth of bacteria on organic matter deposited in sediments from the overlying water column; and (2) in present-day marine basins, typically 18-30% of photosynthetically fixed carbon contributes to the growth of heterotrophic bacteria⁴ and in some highly eutrophic environments up to 80% of photosynthetically fixed carbon is converted to bacterial biomass⁵. This may be of consequence in the light of pre-

liminary evidence for the occurrence of resistant macromolecular components in some bacteria⁶.

The contribution of bacterial biomass to the global carbon budget is of fundamental importance to the biogeochemistry of our planet, having consequences not only for the biogeochemical cycling of oxygen and carbon, but also for palaeoenvironmental reconstructions and the formation of oil-prone sediments. In combination, the arguments of Parkes *et al.*³ and of Ducklow and Carlson⁴ indicate that non-photosynthetically produced bacterial biomass must be significant in the formation of sedimentary organic matter in general. At the other end of the spectrum, the TOC in sediments from the extreme environments of microbial mats may derive mainly from bacterial sources⁷ including photosynthetic bacterial input. In such situations⁸, bacterial markers and the bulk TOC have similar, very heavy, carbon isotope signatures. Thus, Gaia notwithstanding, the jury is still out, and the general conclusion of Hartgers *et al.* that "bacterial biomass may commonly represent only a minor component of total C_{org} in carbonaceous rocks" may be premature.

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HARTGERS ET AL. REPLY — Head *et al.* argue that heterotrophic bacterial biomass production in both the water column^{4,5} and surface sediments³ is important. We agree. However, this by no means implies that bacterial carbon will be ultimately preserved.

Organic matter preserved in sediments represents only a very small portion of organic carbon of biomass, the recalcitrant portion such as algaenans⁹ and cutans¹⁰. In fact, its selective preservation is due to the processes mentioned by Head *et al.* operating in the water column and sediments up to 800 metres below sea floor. Parkes *et al.*³ convincingly demonstrated that the uncharacterized portion of the TOC (non-protein, non-carbohydrate, non-lipid) increases to 80% at 10 m depth in sediments of the Peru Margin. The authors suggested that this unsaponifiable, macromolecular material represents 'bacterial necromass'. The material thus far resisted chemical characterization.

In a more recent paper, Parkes and co-workers¹¹ provide evidence that "bacterial necromass" is a good substrate for other bacteria. This implies, in our view, that bacterial carbon can be recycled as efficiently as the primary photosynthate and is, thus, ultimately completely remineralized if no recalcitrant carbon is present. For example, Saddler and Ward-

law¹² demonstrate that bacterial lipopolysaccharides (LPS) are degraded very rapidly. A substantial bacterial contribution to C_{org} in petroleum source beds can, thus, only be expected if bacteria contain recalcitrant constituents or if remineralization is cut short by temperature, removal of necessary micronutrients, or some similar factor.

So far, the presence of recalcitrant bacterial components in sediments has not been shown, despite the preliminary data concerning the presence of polar resistant organic material in a specific group of pathogenic bacteria⁶. The lack of preserved organic matter in ancient stromatolites derived from bacterial communities in contrast to their contemporary equivalents indicates that such recalcitrant bacterial components are not important. Further, independent carbon isotope analyses of pyrolysates of kerogens of diverse origin are pertinent for two reasons. First, they indicate that contributions from 'hopane-synthesizing bacterial' (cyanobacteria, heterotrophic and methanotrophic bacteria) are minor¹³. Second, they indicate that contributions from resistant algal biopolymers are dominant¹.

In our paper, we concluded¹ that "net contributions of bacterial biomass are

often small" and acknowledged "the undoubted importance of bacterial reworking of sedimentary organic matter". We have avoided any "sweeping generalizations", but we do recommend a sweeping re-evaluation of the belief that bacteria are quantitatively important sources of organic carbon in carbonaceous sediments.

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considered extinct by Rabor survive at Tabunan, as well as the four taxa he found extant⁶. So seven of Cebu's endemic birds survive, twice the number predicted by the species-area relationship. Does this support Budiansky's views that such theoretical predictions are incorrect, and that deforestation does not cause species extinctions?

It does not. Cebu's remaining endemic birds survive in only 15 km² of forest. The latest Red Data Book⁷ lists the island's two endemic species, the Cebu flowerpecker and the black shama (*Copsychus cebuensis*), as 'threatened'. The same criteria⁷ indicate that we should also consider Cebu's five extant endemic subspecies to be 'threatened'. Thus all seven of Cebu's remaining endemic taxa have at least "a high risk of extinction in the medium-term"⁷. This is considerably more serious than the prediction of the species-area relationship that 3.3 species should survive. So Cebu's endemic birds are indeed "committed to extinction"¹. Our discoveries thus herald both good and bad news.

The good news is that there is still hope, even for species written off as extinct like the Cebu flowerpecker. If this rediscovery is possible, surely other 'extinct' species survive elsewhere. The bad news is that we have no breathing space. To save the Cebu flowerpecker and the island's other endemic taxa will require considerable effort. The Philippine Wetland and Wildlife Conservation Foundation Inc. has initiated a project for conservation on Cebu, but it is very short of money. Unless this situation changes in the immediate future, the Cebu flowerpecker will rejoin the list of birds that have become globally extinct, with no hope of rediscovery.

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Extinction and conservation on Cebu

SIR — Heywood *et al.*¹ argue that predicted extinction rates are higher than those observed because of the time lag before we lose species "committed to extinction". Budiansky² suggests instead that the predicted extinction rates are "simply wrong". Our ornithological fieldwork on the island of Cebu, in the Philippines, supports the argument of Heywood *et al.*

Cebu retains 15 km² of dipterocarp forest, 0.3% of its original cover³. In the 1950s, Rabor⁴ failed to record nine of the ten bird taxa then considered endemic to Cebu. He concluded that deforestation had caused the extinction of these birds. Dickinson *et al.*³ considered an additional four taxa to be endemic, of which three are extant. Nevertheless, Cebu appeared to be a textbook application of the species-area relationship to predict extinctions following habitat destruction⁵. We can rewrite $S = c(A)^{0.25}$, where S is the number of taxa, A the area and c a constant, as $S_{new} = S_{original} (A_{new}/A_{original})^{0.25}$. For Cebu, $S_{new} = 14(15/5,088)^{0.25} \approx 3.3$, matching Rabor's finding that four out of 14 endemic taxa had survived.

In 1992, we discovered the Cebu flowerpecker (*Dicaeum quadricolor*), considered extinct since 1906 (ref. 3) in a tiny (2 km²) forest patch at Tabunan⁶. We have also found that two of the subspecies



Very little rainforest remains in the Philippines.

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Dining with Julesz

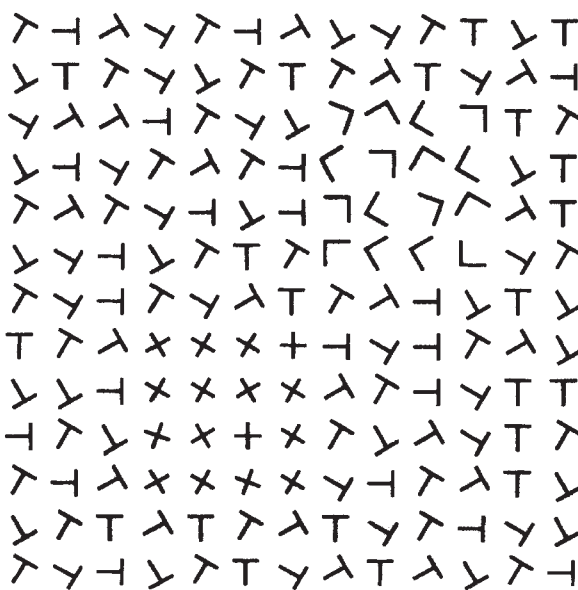
Jeremy M. Wolfe

Dialogues on Perception. By Bela Julesz. MIT Press: 1994. Pp. 276. \$49.95, \$33.50.

Dialogues on Perception is an invitation to get to know Bela Julesz. At present, you cannot escape his influence, even if you do not know his name. Every shopping mall and gift shop bears witness to what is probably his greatest contribution to vision research, the random dot stereogram. The version in the shops is known as an autostereogram. These are the pictures of textured patterns that invite you to cross or uncross your eyes in order to see a ghostly, three-dimensional image emerge from the flat pattern on the wall. They often feature rarefied prose about getting in touch with other planes of consciousness. In fact, when you see the three-dimensional forms, you are getting in touch with binocular stereopsis, the ability to extract depth information from small differences between the images in the two eyes. To see these differences, hold your finger in front of this page, look with the right eye alone and position the finger so it appears just to the left of any word. Now, without moving your finger, look with the left eye alone. The finger now appears to the right of the word. The difference between the two monocular views is binocular disparity.

Our ability to use binocular disparity as a cue to depth was uncovered by C. Wheatstone in 1838, who reported his findings in *Philosophical Transactions of the Royal Society*. For the next 120 years, it was generally assumed that stereopsis added depth to monocularly visible items such as fingers. Then, in the early 1960s, Julesz invented the random dot stereogram and proved that the monocular items were not needed. Purely stereoscopic or 'cyclopean' forms could be seen. Julesz fled Hungary when the 1956 rebellion failed (The Hungarian name is pronounced 'you-less'). He landed at Bell Labs in Murray Hill, New Jersey. From his work as a radar engineer, Julesz knew about camouflage and he knew that stereopsis was one way to defeat it. For example, if the enemy covered its tanks with hay and stuck them in the middle of a hay field, they were hard to spot from the air. But if you took two pictures of the hay field from two different positions as you flew over, you could show one picture to one eye and the other to the other eye. In this stereoscopic view, the tanks would be seen as bumps standing above the field, their camouflage ruined by binocular vision. In the lab,

Julesz used one of the early high-speed computers to show that stereopsis could defeat perfect camouflage. He filled the image in one eye with a random pattern of dots. The other eye saw the same pattern but with a region of dots shifted to the right or the left. Each image alone looked like a formless mass of dots. Seen stereoscopically, however, a sharply defined region of shifted dots appeared to lie in front of or behind the plane of the image.



Find the regions made of 'L's and of '+'s.

Autostereograms work in a similar way. The random background pattern is repeated like a pattern of vertical bars. One eye fixates one 'bar' while the other eye fixates the neighbouring bar. Slight differences in the pattern in adjacent bars give rise to the depth effect (C. W. Tyler and M. B. Clarke, *Proceedings of the Society of Photographic and Illuminating Engineers* 1256, 182–197; 1990).

Julesz's contributions to the study of stereopsis are discussed in *Dialogues on Perception*, but it may come as a surprise that he does not consider the random dot stereogram and its associated science to be his most important contribution. That position he reserves for his work on "textons", his proposed building blocks of texture perception. The figure shows one example of stimuli that Julesz and others have used. In this texture, regions of 'L's and '+'s are embedded in a larger field of 'T's. All of these elements are made of two lines of equal length at right angles to each other. But although all the items are similar, the '+'s segregate into a separate

region whereas the 'L's must be found individually with attentional scrutiny. Apparently, the intersections in the '+'s make them 'preattentively' visible on a background of 'T's. Julesz is one of the founders of the modern study of preattentive processing and remains one of its most active investigators.

In *Dialogues on Perception*, Julesz explains why stereopsis and preattentive processing are worthwhile intellectual pursuits. The book's structure is unusual. Both parties to the dialogues mentioned in the title are Julesz — the 'A' voice is the public Julesz while the 'B' voice is a critical Julesz who tries to deflate A's "unnecessary trappings of human vanity" (p. xviii) and to keep the conversation moving. The result is very much like a series of dinner conversations with Bela. As you can't talk back to the book, you have to imagine one of those situations where you feed an opening line to an interesting person and then sit back to listen, tossing in a comment every so often to keep the dialogue going. "So, Bela, what is new?" you might ask. The result might be Dialogue Twelve, "Recent findings with my co-workers". You wouldn't expect tables of data over dinner and you don't get them here. If you are interested, your companion will tell you where to look. Julesz provides an interesting annotated bibliography covering all of his work to date. There are 14 dialogues in the book. You can imagine that if you were to eat with one person every night for two weeks, you might hear the same story more than once. So, for example, the core of texton theory makes several appearances (the

most comprehensive in Dialogue Nine). You would hope for a steady flow of stimulating ideas and the occasional *bon mot*. These the dialogues provide. I particularly liked Julesz's one-line epigraph for behaviourist psychology: "Psychology without consciousness is like math without infinity" (p. 146) — possible, but not very interesting. Take this book in dialogue-sized bites. Endeavour to hear it in a rich Hungarian accent. It is the next best thing to dinner with Bela. □

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■ MIT Press also recently published *Cognition and the Visual Arts* by Robert L. Solso, an accessible and abundantly illustrated account that relates data on how humans perceive, process and store visual information to the viewing and interpretation of art. Price is \$39.95, £35.95.

Flash Gordon and other scientists

Walter Gratzer

From Faust to Strangelove: Representations of the Scientist in Western Literature. By Roslynn D. Haynes. *Johns Hopkins University Press*: 1994. Pp. 417. \$55, £45.50 (hbk); \$19.95, £16.50 (pbk).

ONE morning not long ago, commuters arriving for work in London were accosted by parties with clipboards and invited to name the most famous living scientists they had heard of. Only three names came up and none belonged to scientists altogether alive: they were Albert Einstein, Alexander Fleming and Stephen Hawking. Einstein and Fleming live on as beatific images, the creations of newsmen and film directors. Hawking is the romantic icon of today. Churchill, asked whether the aged Stanley Baldwin was still alive replied: "Not dead, but the candle in that great turnip has gone out". In Hawking it is the light glowing so fiercely in the inert and ravaged form that has captured the public imagination (certainly not his views on gravitation).

Roslynn Haynes would argue, I surmise, that Einstein, Fleming and Hawking have made such an impression on the tribal consciousness because they conform rather closely to archetypes of the scientist foisted on us by literary tradition. Her book encompasses fictional representations of scientists — broadly interpreted to include engineers, inventors, doctors and psychiatrists — from the birth of modern science to our own day. It is an ample work and the scope of Dr Haynes's reading is prodigious. Starting from the alchemists and the Faust legend, she progresses to Francis Bacon and the early utopias and dystopias. The emergence of the amateur 'virtuosi', as they were called, when the pursuit of knowledge of an undemanding kind became a fashion, turned science into a target for mockery by Ben Jonson and his contemporaries.

Good order was re-established with the entry of Newton (who rates a chapter to himself by reason of his appearance in so much of literature). Newton's achievement was fully recognized by his compatriots and became indeed a subject of patriotic outbursts, such as this from a verse, disinterred by Dr Haynes:

*To thee, great Newton! Britain's justest pride,
The boast of human race.*

But with time this ardour was tempered by unease at the assumptions of superiority that many discerned in the champions of science, and the late seventeenth and early eighteenth centuries saw an ebullition of satire at their expense. This was the time of Pope's *Dunciad* and of Swift and *Gulliver's Travels* (no mention oddly of Defoe), to be followed a little later by the fulminations of Blake. The generally bad press that scientists endured intensified with the rise of the romantic movement and especially the eruption onto the literary scene of Frankenstein (a more complex figure, however, as Dr Haynes shows, than the villain of the Hammer horrors); but although Keats could talk of science unweaving the rainbow, Wordsworth and Coleridge were captivated by the

with his scientific friends, of a utopian vision of a world set to rights by scientists, gives his heroes (and villains) authentic utterance.

Dr Haynes believes that an unflattering picture of the scientist came once more to the fore as the implications of what science had wrought at Los Alamos sank in. Oppenheimer's sombre reflection that physics had lost its innocence — that physicists, as he put it, had known sin — and Fermi's much-quoted maxim that what is "technically sweet" one does, regardless of the consequences, raised the public apprehension of the new monster that science had liberated. A dismal millenarianism replaced belief in progress. As Woody Allen put it, progress was all right, it just went on too long. A third of all horror films, Dr Haynes tells us, have a scientist for a villain.

Mary Evans

But here, it seems to me, her logic falters: what options are there for a horror-film director in search of a villain to freeze the giblets of his audience? A plumber perhaps, who will cause a blockage in the city sewers? Or maybe a dentist? But the imagination fails. Is the evil genius with whom James Bond perpetually grapples a scientist? Or do the villains who train their death-rays on the spaceship in every issue of the *Boy's Own Paper* or *Hotspur* between the wars, only to be thwarted by the intrepid venturers (usually English schoolboys), embody the public perception of the man of science? I doubt it. Dr Haynes's evidence groans a little under the weight of interpretation.

The public perception, she writes, is that scientists "are either too ignorant of consequences or too morally flawed to be entrusted with power; hence their attempts to transform nature are doomed to disaster. The enormous box office success of the film *Jurassic Park* (1993) suggests that this view is still widely held." This too I doubt, nor does it explain why the dinosaur gallery in any natural history museum has always been such a draw.

But Dr Haynes is merciful and spares us the tortures of literary analysis: there is no intertextuality or metanarrative and only a little deconstruction. She writes agreeably and her scholarship is eclectic, for she has pursued her quarry even through the steamy pages of Bram Stoker, not to mention Flash Gordon, who, you may remember, wages an unsleeping struggle against Ming the Merciless from the planet Mongo. When Flash (in the shape of Gene Autry) succeeded in destroying the atom furnace, which was the instrument Ming had in mind when he declared that "Radioactivity will make me Emperor of the Universe", he revealed to an open-mouthed populace, so Dr

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Marlowe's Faustus (1636): seeker of "Unlawful things/ Whose deepness doth entice such forward wits/ To practise more than heavenly power permits."

discoveries of the time — Coleridge attended Humphry Davy's lectures at the Royal Institution — and absorbed scientific imagery into their verse.

In the Victorian novel evil and virtuous scientists alternate. Such writers as Mrs Gaskell and Charles Kingsley knew and admired Darwin and were on principle well-disposed towards the scientific enterprise. Others saw only demons; Dr Haynes gives many examples, but she omits perhaps the most extravagantly malign character of all — a product of his creator's obscurantist beliefs — the sinister vivisectionist, Dr Benjulia, in Wilkie Collins's *Heart and Science*.

The scientist as fearless explorer-hero begins to assert himself in the scientific romances of Jules Verne, Conan Doyle and many European writers of the period (although one of the most notable of the futurists, the Russian, Tsiolkovsky, does not appear among Dr Haynes's chosen). Thomas Edison, a venerated magus to a generation of Americans, surfaces as the hero of a series of stories and novels. And then of course H. G. Wells, the proponent,

Haynes asserts, the power of the new forces that Rutherford had unleashed in Cambridge and expounded in his little book *The Newer Alchemy*.

I enjoyed Dr Haynes's book and most of all I am grateful to have been introduced to so many works of fiction about science and scientists, serious and frivolous, of which I was ignorant. I take issue with Dr Haynes on her denunciation of the scientists of the Manhattan Project for betraying their responsibility to society. If she is still searching for a real *trahison des clercs*, she might direct her attention to the new biologists, especially those who are even now trying to make patents of the genes that evolution gave us, for nothing but lucre. □

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Reading the mind of God

Colin Tudge

The Development of Biological Systematics: Antoine-Laurent de Jussieu, Nature and the Natural System. By Peter F. Stevens. Columbia University Press: 1994. Pp. 616. \$50, £65.

In the eighteenth century, the overriding concern in science, as in the arts and all philosophy, was with order; and in science, with the notion that behind the order lay natural law that was there to be discovered. Appropriately, although the seventeenth century set the ball rolling, the eighteenth truly began the modern disciplines of taxonomy and systematics: the arts and science of naming living organisms and arranging them into groups that are not only tidy but also correspond with the presumed but as yet undiscovered patterns of nature.

A seminal figure now known to all the world was the Swedish botanist Carolus Linnaeus (original name Carl von Linné; 1707-78). He began formulating his taxonomic system as early as 1730, published *Systema Natura* in 1735 and made his lasting contribution to taxonomy with his binomial system for naming organisms in 1749. At least of equal significance, however, was a French botanist who is now known only to professionals: Antoine-Laurent de Jussieu, who was born the year before Linnaeus devised his binomial system and lived until 1836 — thus seeing seven and a half decades of extraordinary upheaval, intellectual, technical and political. Jussieu's *Genera plantarum secundum ordines naturales disposita* was published in 1789, virtually as the Bastille

was falling. It describes all the plant genera then known and places them in order, and effectively set the tone of systematics until the twentieth century. Peter Stevens has done the history of the subject much service by providing both a new commentary on this neglected scientist and the translations of Jussieu's early works which fill the 117 pages of Appendix 1.

But the notion that classification should reflect natural order begs many questions and raises deep practical issues. Nowadays, almost 140 years after Charles Darwin's *Origin of Species* of 1859, most biologists take it for granted that natural 'order' springs from genealogy, which classification should reflect. Organisms should ideally be grouped together only if they can all be shown to share a common ancestor. But ideas on evolution were at best haphazard in the eighteenth century and ideas of order had to find inspiration from elsewhere. If this 'elsewhere' was presumed to be 'the mind of God', the task was still to unravel the rules by which God was operating.

Jussieu derived orderliness from the premise — held also by others, but emphasized by him — that nature is 'continuous'; that is, living plants between them formed an uninterrupted series, and each species in the series graded by minute degrees into the next one along. Nature makes no jumps: *natura non facit saltus*. Allied to this supposition, though not vital, was the idea of 'plenitude'; that all conceivable life forms were manifested somewhere. If there were gaps, it was only because the intermediary forms had not yet been found, but such was the frenetic pace of exploration in the late eighteenth century that Jussieu was confident that the gaps would soon be filled. However, Jussieu did not envisage the series as a one-dimensional *scala naturae* but as a kind of map, with the different groups analogous to countries. Thus France is recognizably discrete from Germany even though the land mass is continuous; but the presence of Alsace reminds us that the distinctions are not absolute. Stevens points out, however, that the idea that nature is 'continuous' is not logically compatible with the idea of natural groups at all, as the barriers between the groups, like political boundaries, exist only in the minds of the observers. Similarly, Stevens questions the modern conceit that the groups of eighteenth-century systematists were supposed to reflect some Aristotelean 'essence'. The idea of discrete

'essences' does not fit easily with the notion that each group grades seamlessly into the next.

In practice, however, the idea that living forms made a continuous 'landscape' quickly gave way to one of a reticulum — a map with contacts between recognizable groups but also with gaps. Finally the notion emerged that is still extant: that the relationship between the different living groups could best (or at least most safely) be represented as an archipelago, with clear gaps between them.

In principle, Darwin's ideas of evolution seem to resolve all these dilemmas: the all-too-perceptible gaps are the gaps

between the twigs of an evolutionary tree, and the intermediates are common ancestors that may be long extinct and may or may not have been discovered. We might expect, therefore, that that systematics would have changed radically after Darwin. But in fact it did not, not least because the discipline was driven by its own methodology — by the techniques that Jussieu and others had developed, on a somewhat arbitrary theoretical base, to solve practical problems. However one conceives 'relationship', the seminal difficulty is always to decide

which organisms are really related to which. Now that systematists acknowledge evolution, they seek homology: characters in different organisms that are similar because they descended from a common ancestor, as opposed to 'homoplasy', where apparently similar characters have evolved quite separately in different lineages.

There are well-established criteria by which to distinguish homology from homoplasy but there is still no royal road to truth. Modern systematists have many more specimens to look at than Jussieu did, including a vast range of extinct types; and the 'characters' they explore include those of molecules and not merely of external features, which Jussieu preferred, or of tissues and cells, as became more fashionable in the nineteenth century. Because the moderns have so many features to assess, too, they can supplement their analyses with statistics. Yet, like Jussieu, they must still use their human judgement to decide which features truly reflect relationship and which are fortuitous. Jussieu recognized a hierarchy of importance among characters. Thus he felt that the number of seed-leaves (cotyledons) was most instructive, then flower structure and so on; an

IMAGE
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Laurent de Jussieu. Credit: Mary Evans

arbitrary judgement that he sometimes applied eccentrically, but is essentially considered to be correct and is still adhered to.

Like the old-timers, too, modern taxonomists must still decide whether their classifications should rigidly reflect real relationships — in which case they can become highly intricate and cumbersome — or should be more practical. Jussieu pondered this: “Botany is not worth taking our time unless it can become useful for man ... [it] should be worthy of occupying the attention of botanists who would wish to join with the title of Savant that of worthy Citizen”. He wrote that in 1774. It

is intriguing to see the loaded word ‘Citizen’ thus emphasized at that time.

The Development of Biological Systematics would have wider appeal if Stevens had spread himself into the seventeenth and twentieth centuries, to show how the eighteenth-century discipline arose and how eighteenth-century concerns have been superseded, or remain with us. But this is exemplary scholarship which, as is the task and purpose of history, exposes the roots of ideas that we too readily take for granted. □

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matter, Pursell readily admits that he does not always examine the background, operation and consequences of the technologies he discusses. Yet his analyses of the fundamentally reflexive relationship between culture and technology are invariably interesting and provocative. Such controversial topics as changes in the nature of invention, the perception of space and time, warfare, mass production and scientific management, private decency and public health, information access and dissemination and, not least, the science–technology nexus are presented in an intriguing if not necessarily original light. Unlike Snow in his attempt to be sympathetic, Pursell makes it clear that engineering is not simply applied science.

One of Pursell’s greatest strengths is his recognition of the many ironies of technology’s evolution, of developments that had consequences far different from and far larger or smaller than those expected by their originators, whether for better or for worse. Another strength is his repeated attention to gender issues, be they the composition of the workforce and the invention community or the language and metaphors used to describe tools and machines and the societies using them.

White Heat has 80 excellent illustrations, all of them appropriately identified. For whatever reason, however, only some of the many quotations of, or references to, others’ ideas running throughout the book have footnotes. In the absence of a bibliography, this seemingly arbitrary system of citation is especially unfortunate. Despite this lapse, *White Heat* is a genuine contribution to technology studies that deserves a wide readership. □

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Technology everywhere

Howard P. Segal

White Heat: People and Technology. By Carroll Pursell. BBC Books/University of California Press: 1994. Pp. 224. £14.99, \$18 (pbk).

THIRTY-five years after C. P. Snow’s *The Two Cultures and the Scientific Revolution* first appeared, it has yet to be widely appreciated that Snow identified engineering and technology generally as a third culture separate from the two he focused on, science and the humanities. In passages of his book too rarely cited, he acknowledged that scientists can be as patronizing toward engineers and other technical experts as they can towards any humanists. As Snow put it, “Pure scientists have by and large been dim-witted about engineers and applied science. They couldn’t get interested. They wouldn’t recognise that many of the problems were as intellectually exacting as pure problems, and that many of the solutions were as satisfying and beautiful” as those produced by scientists. Instead, scientists invariably assumed that “applied science was an occupation for second-rate minds”. As Snow understood but sadly didn’t stress sufficiently, technology is an intellectual as well as a mechanical enterprise, and those who engage in it are not the servants of scientists. If, as some have characterized the fundamental difference, science is discovery while engineering is design, the activity of design is itself highly creative.

The identification of technology as an intellectual and creative pursuit has been a principal contribution of the field of the history of technology founded by Melvin Kranzberg in the late 1950s and early 1960s. It is no accident that the leading journal in the field, the one Kranzberg established and edited for many years, is called *Technology and Culture* (University of Chicago Press). Ironically, the journal began because historians of science would not grant sufficient space in their own

journals and professional meetings to what at the time were dismissed as boring and insignificant ‘nuts and bolts’ analyses.

A leading historian of technology himself, Carroll Pursell is sensitive to the prejudices and misconceptions of not just scientists and historians of science but also the general public. Much of his earlier research and writing has illuminated public attitudes and public policy concerning technology. His new book draws on his expertise to offer insights into the evolution of technology over the centuries.

White Heat is intended to serve as a companion volume for the BBC television series of the same name but is not, says Pursell, a substitute for it. Alas, because the series is such a disappointment to those of us who had sought a satisfactory replacement for James Burke’s often disjointed and overly glitzy “Connections” series, the book should indeed be judged on its own merits. Although Pursell’s eight chapters do correspond to the series’ eight segments, the former vary sufficiently from the latter that one can profit from skipping the series altogether and concentrating on the book alone.

Citing the *Oxford English Dictionary*, the book defines ‘white heat’ as both the temperature at which metal emits a white light and a state of intense passion or activity. Forging tools by fashioning metal in this way represents technological progress — and progress in the mechanical and intellectual senses alike. If Pursell is hardly an uncritical apologist for technological advance and *White Heat* hardly an example of Herbert Butterfield’s Whig interpretation of history, the sheer pervasiveness of technology in the contemporary world and contemporary culture is one of Pursell’s key points.

That *White Heat* is intentionally presented in “something of a collage rather than a linear narrative” may limit its usefulness as a text for courses or as a primer for interested general readers. For that

New in paperback

What if the Moon Didn’t Exist? Voyages to Earths That Might Have Been by Neil F. Comins. HarperPerennial, \$13. “What if this book didn’t exist?” asked Tobias Owen in his review in *Nature* **368**, 412 (1994). His conclusion: “We would miss the author’s playful speculations about alternative Earths, about the ways in which our planet might have evolved if circumstances surrounding its origin and early history had been different. But we would be spared a lot of misinformation”.

Time Machines: Time Travel in Physics, Metaphysics, and Science Fiction by Paul J. Nahin. AIP (distributed by Oxford University Press), £12.95. “The research that has gone into this book is impressive (Ian Moss, *Nature* **365**, 24; 1993).

Connecting landscape patterns to ecosystem and population processes

Peter Kareiva & Uno Wennergren

Spatially explicit ecological models offer numerous surprises, ranging from intricate tapestries of spatial patterning to widespread extinction when roads or deforestation fracture a landscape. Many of the gloomier examples involve thresholds, the approach to which would be hard to anticipate with standard census data. The challenge is in making a constructive management tool from these abstract harbingers of ecological disruption.

If you look out of an aircraft window while it is flying, you are likely to see quilt-like mosaics of farmland and woodland, urban sprawl and meadows, or clearings and old growth forest. These chequered landscapes reflect massive amounts of habitat destruction, which is so rampant that conservationists throughout the world are concerned about protecting locally scarce parcels of pristine vegetation^{1,2}. Meanwhile, the consequences of landscape change are generating a great deal of interest among theoretical ecologists, who are beginning to use spatially explicit models to predict the impact of different landscape configurations on plant and animal populations. The phrase 'spatially explicit' refers to the fact that the models keep track of the exact positions of plants and animals, so that higher-level processes such as productivity or pestilence, can be related to landscape geometries. The 'effects' that are of interest include bizarre population eruptions³, loss of biodiversity⁴ and the stabilization of otherwise fluctuating dynamics⁵⁻⁷. Links are even being forged between seemingly abstract models and the concerns of land managers, with the underlying assumption that if we understand how landscape pattern determines population or ecosystem dynamics, we can better manage our lands⁸. Given the practical appeal of these investigations, it is time to ask whether any general principles are emerging from the explosion of spatially explicit theory. Here we examine two distinct types of spatial models: (1) those that depict the world in terms of occupied and unoccupied patches and offer a qualitative picture of overall population trends; and (2) a complementary class that deals with the dynamics of populations within patches, thereby allowing a more quantitative accounting of dynamics.

Extinction thresholds for single species

One of the most powerful metaphors for population dynamics is a series of patches winking in and out of existence like Christmas-tree lights, with the average number of 'on patches' (or extant populations) determined by a balance between extinction and colonization⁹. This view of population processes, called metapopulation dynamics, carries with it several crucial insights. First, metapopulations will involve a shifting mosaic of presence and absence, with patterns of patch occupancy (as opposed to details of within-patch events) providing the key to understanding¹⁰. Second, a population governed by metapopulation processes will be vulnerable to a threshold of habitat availability, below which the metapopulation will descend to extinction because colonization is too infrequent to overcome random local extinctions^{11,12}. For example, if we let v denote the fraction of habitat patches occupied by a species, then a plausible description of temporal changes in 'patch occupancy' is given by:

$$dv/dt = cv(1-v) - ev \quad (1)$$

where c is a parameter reflecting colonization abilities, e is the instantaneous extinction rate, and $(1-v)$ is the fraction of patches that are vacant but potentially occupiable. If a proportion, D , of the original habitat patches is destroyed, then the

colonization component of equation (1) is decremented to $cv(1-v-D)$, such that dv/dt may become inescapably negative for all v . This means there is a threshold value for D , at which point so much habitat has been lost that the species sets off on an inexorable decline to extinction. The extinction of a metapopulation following the removal of too much habitat is conceptually identical to the collapse of a disease epidemic following the removal of too many susceptible hosts¹³, a correspondence that suggests we could learn something about how species persist from recent advances in the theory of host-pathogen interactions¹⁴.

Of course, in strict terms, equation (1) and related metapopulation models are not explicitly spatial because habitat patches and distribution patterns are not assigned to any spatial coordinate; nonetheless, these patch occupancy models imply a spatial world view and have provided a powerful motivation for more explicitly spatial models. For example, Lande¹⁵ modified equation (1) to point out that the demography of northern spotted owls could not be disconnected from the landscape changes associated with logging. Lande's analysis provided impetus to US Forest Service scientists to develop explicit spatial models for spotted owls in which patterns of deforestation directly alter owl success^{16,17}.

Most applied research regarding the dynamics of a single species in complex landscapes entails detailed simulations that move individuals around in landscapes drawn from actual maps of habitat^{16,18}. The use of real landscapes in these simulations can suggest a false exactitude, which disguises ever-present uncertainties about how the animals are actually behaving. For example, although it is straightforward to map vegetation, it is extraordinarily difficult to obtain information on dispersal behaviour. Consequently, questions of model sensitivity^{19,20} and parameter estimation²¹ dominate research that aims to connect landscape ecology to policy.

Biodiversity and extinction patterns

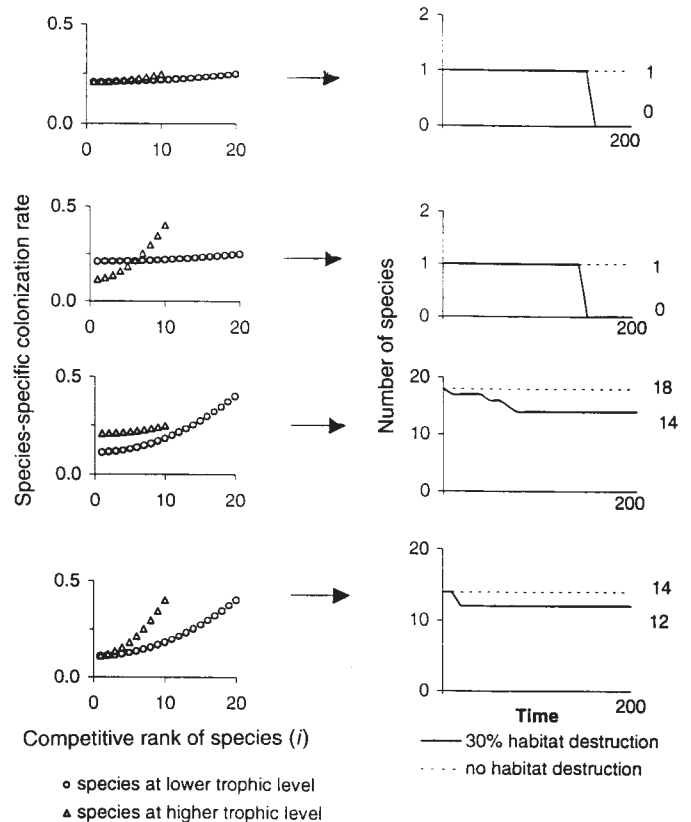
The same metapopulation processes of extinction and colonization that are central to the fate of single species also influence communities of species. Theoreticians have long recognized this^{22,23}, but have only recently used multispecies metapopulation models to investigate the consequences of habitat destruction. In a simple extension of equation (1) to competitive interactions between two species, Nee and May²⁴ point out that removal of habitat can alter patterns of coexistence because it favours organisms with high colonization ability over less mobile, but more competitive species. This portends general declines in biodiversity when too much habitat has been destroyed, because some species may find themselves unable to colonize vacant patches fast enough. The crucial mechanism can be demonstrated by considering just two species, where v_i is now the fraction of patches occupied by species i , D is the proportion of habitat patches permanently destroyed, c_i is the rate of colon-

FIG. 1 The effect of differentiated colonization rates on extinction debt for a two trophic level community described

$$dv_i/dt = c_i v_i \left(1 - D - \sum_{j=1}^i v_j - \sum_{j=1}^{10} p_j \right) - e v_i - v_i \sum_{j=1}^i c_j v_j - v_i \sum_{j=1}^{10} c_j p_j$$

$$dp_i/dt = c_i p_i \left(\sum_{j=1}^{20} v_j - \sum_{j=1}^i p_j \right) - e p_i - p_i \sum_{j=1}^{i-1} c_j p_j$$

where p_i is the proportion of patches occupied by the i th species of predators, and v_i is the proportion of patches occupied by the i th species of prey, e is an extinction rate, and the c s are species-specific colonization rates. This is the 'metapopulation model' of Tilman *et al.* with a second trophic level added. Like Tilman *et al.*⁴ we assume a hierarchy of competitiveness for both predators and prey, such that species 1 can always colonize and exclude all lower-ranked species sharing its trophic level (species 2, 3 and so on). The graphs on the left depict the colonization rates for predators and their prey. Communities were seeded with 20 prey species and 10 predator species with those colonization rates, and then allowed to come to an equilibrium. At that point 30% of the habitat is destroyed, and we simulate the decline in diversity that follows (time $t=0$ is the time of habitat loss, $D=0.3$) as shown in the graphs at the right (the dashed line represents species richness in the absence of the habitat loss). The above results were obtained with the extinction rate, $e=0.1$ for all species. The mean colonization rate is always 0.23, with it either varying from 0.21 to 0.25 or from 0.11 to 0.40 (this applies to both predators and prey, but for predators, for which there are fewer species, the increments between nearest ranked species are larger). Clearly, differentiation among prey with respect to colonization is a prerequisite for high biodiversity, but once this differentiation exists, the addition of predators does not change the basic pattern, nor does it change the depletion in biodiversity associated with the 30% loss in habitat.



ization of vacant patches by species i , and e_i is the extinction rate for species i within occupied patches:

$$dv_1/dt = c_1 v_1 (1 - v_1 - D) - e_1 v_1 \quad (2)$$

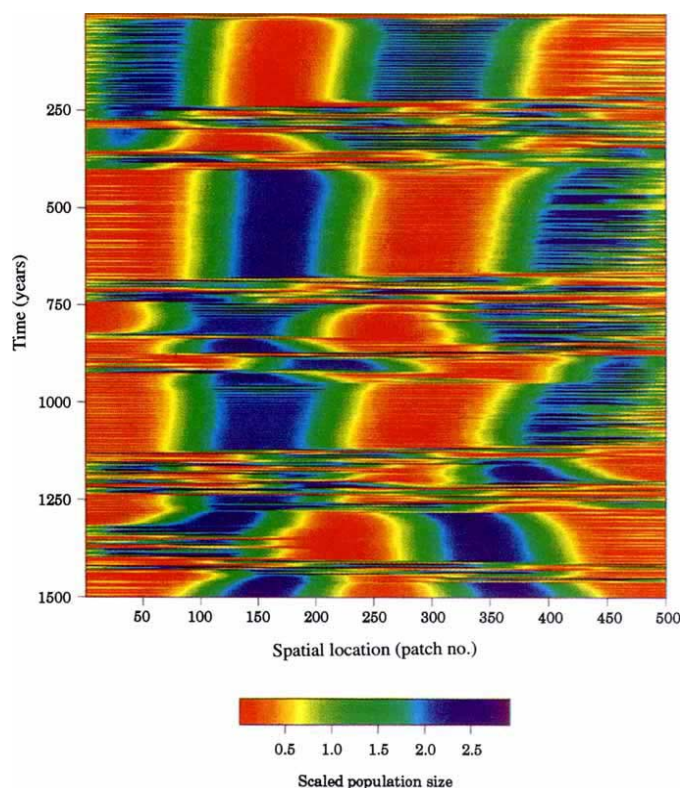
$$dv_2/dt = c_2 v_2 (1 - v_1 - v_2 - D) - e_2 v_2 - c_1 v_1 v_2 \quad (3)$$

with the differences in dynamics for species 1 and 2 arising because we assume a strict competitive hierarchy in which species 1 can colonize and displace patches occupied by species 2, but species 2 cannot colonize patches occupied by species 1. This model allows coexistence as long as the inferior competitor enjoys an advantage of higher colonization rates (c_2 sufficiently greater than c_1), but it is a coexistence vulnerable to habitat destruction, because as D gets large the competitively superior species will be placed at risk. It is a simple matter to generate equally crisp predictions for N -species communities by enforcing a strict competitive hierarchy for all N species (species 1 always displaces species 2, 3... on down to N , species 2 always displaces species 3, 4... and so on and assuming that colonization abilities (c_i values) increase with declining competitive ranks. Using this approach, Tilman *et al.*⁴ show how bouts of habitat destruction are likely to be followed by delayed sequences of extinction, a phenomenon they vividly label the 'extinction debt'. Three characteristics of this extinction debt make it especially disturbing. First, this metapopulation model predicts long time lags before species disappear as a result of habitat destruction. Second, the amount of extinction caused by habitat destruction is an accelerating curve, such that large amounts of initial habitat loss could yield only minor extinction, only to be followed by a sharp upturn in extinction as D gets sufficiently large. Finally, the species most prone to extinction are the superior competitors, those highly successful abundant species we might never guess are at risk. These three features of the extinction debt combine

to imply that prospects for biodiversity are likely to be much worse than they appear: extinctions will accrue exponentially with increasing habitat loss, long time delays will occur before we see evidence of the extinctions, and even common competitively superior species are likely to be at risk (if their dispersal capacity is limited).

Before succumbing to despair, one must realize that the model of Tilman *et al.* is an idealization, with many simplifications that may limit its generality. For instance, although there is no question that poor dispersers are especially vulnerable to habitat fragmentation, it remains to be seen whether there is a widespread negative correlation between dispersal and competitive ability. Second, because the approach embodied in equations (2) and (3) distributes colonists equally among all patches, there is a need to examine more realistic models that incorporate local dispersal. Tilman *et al.*⁴ have taken this step (which precludes simple analytical treatment) and found that if the world is organized into a 'honeycomb' of patches with colonization possible only from one patch to neighbours within a radius of four grid cells of the colonists, then all of the features of extinction debt revealed from their elegantly simplified model, also appear in their spatially explicit formulation. Finally, one needs to ask whether the addition of another trophic level, such as predators or herbivores feeding on the competitors, will radically alter opportunities for an extinction debt. After adding a second trophic level to this model, we have found that their results still hold (Fig. 1). In fact, even if the predators are not differentiated with respect to dispersal ability, as long as their prey compete and exhibit the dispersal-competition tradeoff hypothesized by Tilman²⁵, one observes extensive, delayed extinction following habitat loss (Fig. 1). As a wider variety of multitrophic-level models are explored²⁶, it will be important to determine whether dispersal/competition tradeoffs at the lowest trophic

FIG. 2 Population dynamics over space. The intricate spatiotemporal variation in population density observed under the simple spatially explicit recursion model of $N_{x,t+1} = N_{x,t} \exp[r(1 - N_{x,t})]$ with N being population density at position x and generation t , and the dispersal of young along x assumed to be through a diffusion process such that offspring end up being distributed about their birthplace according to a gaussian distribution with variance z , see Hastings and Higgins³ for details. For this output the parameters are $r=3.25$ and $z=47$. The temporal axis is plotted every other year, because this set of parameters yields roughly two-point cycle. An important lesson from this figure is that the dynamics could never be appreciated by tracking density at any single position for a long time period, or by tracking densities at all positions for a short time period. The equilibrium density here is scaled to be $N=1$, which means that purple represents extreme overshoots of the equilibrium, and red represents extraordinarily scarce organisms relative to their natural equilibrium.



level consistently prevail as the primary determinants of extinction debts.

Of course, community metapopulation models are little more than metaphors, just as equation (1) is little more than a metaphor for a single species in a fragmented habitat. For these models, the representation of biology is so simplified that, at best, they can only point out general possibilities and alert us to dangers. When managing real ecosystems, one needs to ask questions about population dynamics and stability, rather than simply extinction. Models for this purpose must capture more biological detail than presence or absence from idealized patches in an idealized landscape²⁷. Models that couple species interactions with spatially explicit population dynamics represent one of the most exciting areas of theoretical ecology, and an area that is just beginning to be explored²⁸.

Ecosystem and population stability

To examine ecosystem dynamics in altered landscapes, theoreticians rely on models in which the population densities of competitors, predators and their prey, or pathogens and their hosts, are followed through time and space. The focus of these analyses tends to be on either spatial patterning or the degree of fluctuations observed in populations. Fluctuations are especially pertinent if one is worried about crop pests, harvesting fish or wildlife populations, or disease epidemics. Some of the most interesting work involves studies of host–parasitoid interactions using cellular automata models. Here, coexistence is associated with striking spatial patterns that represent tapestries of alternating high and low densities of hosts and their parasites, even though the underlying environment is uniform^{29–31}. If the spatial domain is too small or is fractured by barriers to dispersal (such as highways), then the spatial patterning, and the coexistence it affords may collapse³¹. An especially intriguing result for these cellular automata models pertains to three-species systems, with either two hosts and one parasitoid, or two parasitoids and one host. In such systems, it has been found that competing hosts or

competing parasitoids may coexist by exhibiting a pattern of spatial segregation²⁹. This might not seem notable because ecologists know of many examples of species that occupy different habitats, but this case is fascinating because the environment is uniform, and the segregation is strictly a result of the interplay of local dynamics and dispersal, not of environmental heterogeneity and habitat preference.

A second important theoretical result to emerge from spatially explicit population models is the possibility of extremely long transient behaviour, including alternating sequences of chaos and limit cycle behaviour. Hastings and Higgins³ have shown that even the simplest single species model with density dependence and random dispersal can generate extraordinarily rich patterns of density variation in space that shift unpredictably through time and take thousands of years to settle into any steady pattern of dynamics (Fig. 2). Although the sheer complexity of this spatiotemporal pattern of dynamics is itself intriguing, the pragmatic implications are disturbing. If the presence of a spatial dimension makes transient dynamics so long, and so unsteady, then trend analyses or even model-fitting could be quite unreliable.

Finally, there is the much discussed⁶ capacity of a spatial dimension to stabilize predator–prey dynamics that would otherwise fluctuate wildly. A variety of robust modelling approaches indicate that predator–prey interactions confined to small regions will tend to oscillate off into extinction, but that a game of predatory hide-and-seek, or a linked system of out-of-phase population oscillations, can stabilize these interactions^{5–7,30,32}. Curiously, empirical documentation of these widely accepted ideas is almost totally lacking⁶. However, theory may tell us why experimentalists have such a hard time verifying this stabilizing mechanism, the spatial and temporal scales at which the phenomenon would be clearest are well beyond the scales at which experimental ecology traditionally operates. For instance, cellular automata models suggest a stabilizing effect only on the scale of landscapes orders of magnitude larger than the lifetime disper-

sal of the organisms under study³³, which is a scale rarely studied by population biologists.

Emerging principles

At first glance, the models we have reviewed might seem to offer nothing more than a bewildering assortment of specific results, all unrelated to one another. However, clarity can be gained by focusing on the practical messages needed by resource managers.

- Species that act as metapopulations live with a threshold requirement for habitat, below which they face inevitable extinction (long before all of the habitat has been removed).
- Destruction of habitat can cause dramatic loss in biodiversity that is long delayed, nonlinear and conspicuous only after substantial habitat disappearance with surprisingly negligible effects has occurred. This means that monitoring programmes and trend analysis may offer a false sense of security that hides the risks of continued habitat loss.
- The details of how habitats are arranged and how organisms move between them can mitigate the above risks, and dictate whether species persist or go extinct, or whether populations fluctuate wildly or remain stable. Management options that emphasize dispersal rates or the deployment of habitat can help to maximize the benefit of conservation efforts.
- The mere fact that species interact in a spatial world makes possible an extraordinary richness of possible distributional patterns and population dynamics. Exactly what patterns or dynamics we observe will depend on how long a time period and over how large an area we collect our data. Local census programmes of limited duration may entirely misrepresent the true population dynamics at play, and could mislead our management efforts on behalf of endangered species²⁰.
- If we assume that the building of highways and the spread of human populations fractures habitats into relatively isolated parcels, then one of the impacts of landscape change may be the elimination of opportunities for species coexistence through spatial complexity.

Translating the theory into guidelines

Landscape modelling began as an arcane academic pursuit but, like much of ecology, the pressure of environmental problems has forced these investigations into the limelight of public scrutiny, even to the point of becoming a focus of legal battles regarding national resources policies³⁴. One critical test for ecological science will be whether it can profitably use the insights that spatially explicit models generate to solve practical problems. We suspect it can, as long as resource managers do not use the

models in a superficial way, and as long as policy makers realize that maps of fragmentation and habitat structure alone do not lend much insight without hard data on how species disperse and interact with other species. Current biodiversity mapping projects that use geographic information systems³⁵ will be most useful when they are used to look at dynamics, as opposed to static snapshots, and are connected to mechanistic theories that predict population dynamics as a function of landscape attributes. Ecology has a history of being misled by purely observational analyses of patterns bereft of dynamics³⁶, a mistake to be avoided in the application of landscape analysis to conservation efforts.

A final worry is the lure of an ingenious model. It is natural to be fascinated by pictures of elaborate lattices or spirals that appear on a computer screen. Similarly, the idea of extinction debts and of sudden switches from constant populations to chaotic dynamics appeal to our love of surprise. To imbue such theoretical curiosities with practical implications, modellers often use vague phrases like 'the observed patterns are consistent with the sorts of complex dynamics we often see in nature'. We need to remember yes, but these fancy results may have little to do with the real reasons underlying the collapse of our ecosystems and declining biodiversity. It could all be so much simpler: species are steadily lost in direct proportion to habitat destruction (without hidden thresholds that creep up on us); uneven distributions in plants and animals simply reflect underlying habitat heterogeneity (not crystal lattice structures); and sudden changes in population trends arise because of an obvious human disturbance (not a transient shift caused by highly nonlinear dynamics). For mathematical elegance the first generation of spatially explicit models has asked what dynamics and patterns arise in uniform environments, now we need to ask how habitat heterogeneity alters these patterns. For example, how does spatial variation in disease transmission alter the likelihood and severity of epidemics?^{14,37}

The pressing theoretical questions for the future will involve studies that ask how external forcings such as climate change, environmental gradients and habitat loss interact with metapopulation dynamics and spatial pattern formation to govern ecosystem dynamics³⁸. The issue is not whether landscape models describe ecosystems, but whether they add substantially to the understanding we would arrive at in the absence of a spatially explicit perspective. Theory tells us they should, but only data can tell us whether they do. □

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Structure of NF- κ B p50 homodimer bound to a κ B site

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The 2.3-Å crystal structure of the transcription factor NF- κ B p50 homodimer bound to a palindromic κ B site reveals that the Rel homology region folds into two distinct domains, similar to those in the immunoglobulin superfamily. The p50 dimer envelopes an undistorted B-DNA helix, making specific contacts along the 10-base-pair κ B recognition site mainly through loops connecting secondary structure elements in both domains. The carboxy-terminal domains form a dimerization interface between β -sheets using residues that are strongly conserved in the Rel family.

THE transcription factor NF- κ B was first identified as a protein with DNA-binding activity specific for the 10-base-pair (bp) κ B site in the immunoglobulin κ light-chain enhancer of B lymphocytes¹ and is now the heterodimeric prototype of a growing family of transcription factors that share high sequence identity over a 300 amino-acid 'rel homology region' (RHR, Fig. 1a, b; refs 2, 3). Members of this family, which include p50, p52, p65, c-Rel, v-Rel, RelB, and the *Drosophila* proteins, Dorsal and Dif, exist as homo- and heterodimers that bind to κ B sites in the enhancer regions of a large number of target genes, most of which are involved in cellular defence mechanisms and differentiation^{3, 13}. The RHR, found at the amino terminus of these proteins, is responsible for protein dimerization, DNA binding, and nuclear localization, whereas the highly variable C-terminal domains (not found in p50 or p52) are probably responsible for transactivation (Fig. 1a; refs 2–6). DNA binding requires the entire RHR, unlike most prokaryotic and eukaryotic transcription factors where much smaller DNA-binding domains often confer full specificity and binding affinity for the target.

NF- κ B proteins have been found in all mammalian cells tested. In most cells the proteins are cytoplasmic, in an inactive complex with inhibitory proteins from the I- κ B family^{14, 15}. As shown in Fig. 1a, the C-terminal regions of most Rel proteins contain transcriptional activation domains. In contrast, p50 and p52, which contain no C-terminal activation domain, are synthesized as larger precursors that contain C-terminal I- κ B sequences that inhibit their nuclear translocation (Fig. 1b; refs 7, 8, 11). These precursors are subsequently processed through a ubiquitin–proteasome conjugated pathway to produce mature p50 and p52¹⁶. The p50 and p52 homodimers both appear to modulate transcription by competing with other NF- κ B dimers for κ B site binding³. Nuclear localization, proteolytic processing, phosphorylation and the combinatorial diversity of dimers are all mechanisms that control NF- κ B-mediated responses. The I- κ B proteins are also tightly regulated, with κ B-mediated transcriptional control and phosphorylation playing important roles¹⁷.

Here we report the 2.3 Å crystal structure of the RHR from mouse NF- κ B p50 bound as a homodimer to an idealized palindromic κ B target. The structure of the complex, shown in Fig. 2, reveals a new mode of DNA binding by the Rel family of proteins. The RHR folds into two domains. The C-terminal domain (domain 2), containing a core β -sandwich structure shared by the immunoglobulin superfamily (Fig. 1d), is responsible for dimer formation. The dimer interface is formed by the face of a three-stranded β -sheet packing against the same sheet in the opposing subunit (Fig. 2d, e). Both domains and the segment that connects them form a continuous sequence-specific

DNA-binding surface by contributing 10 loops (5 from each subunit) that fill the entire major groove of the target DNA. The binding surface of the N-terminal domain (domain 1) is augmented by a variable length α -helical segment that forms strong contacts with the minor groove near the centre of the κ B element.

Structure determination and refinement

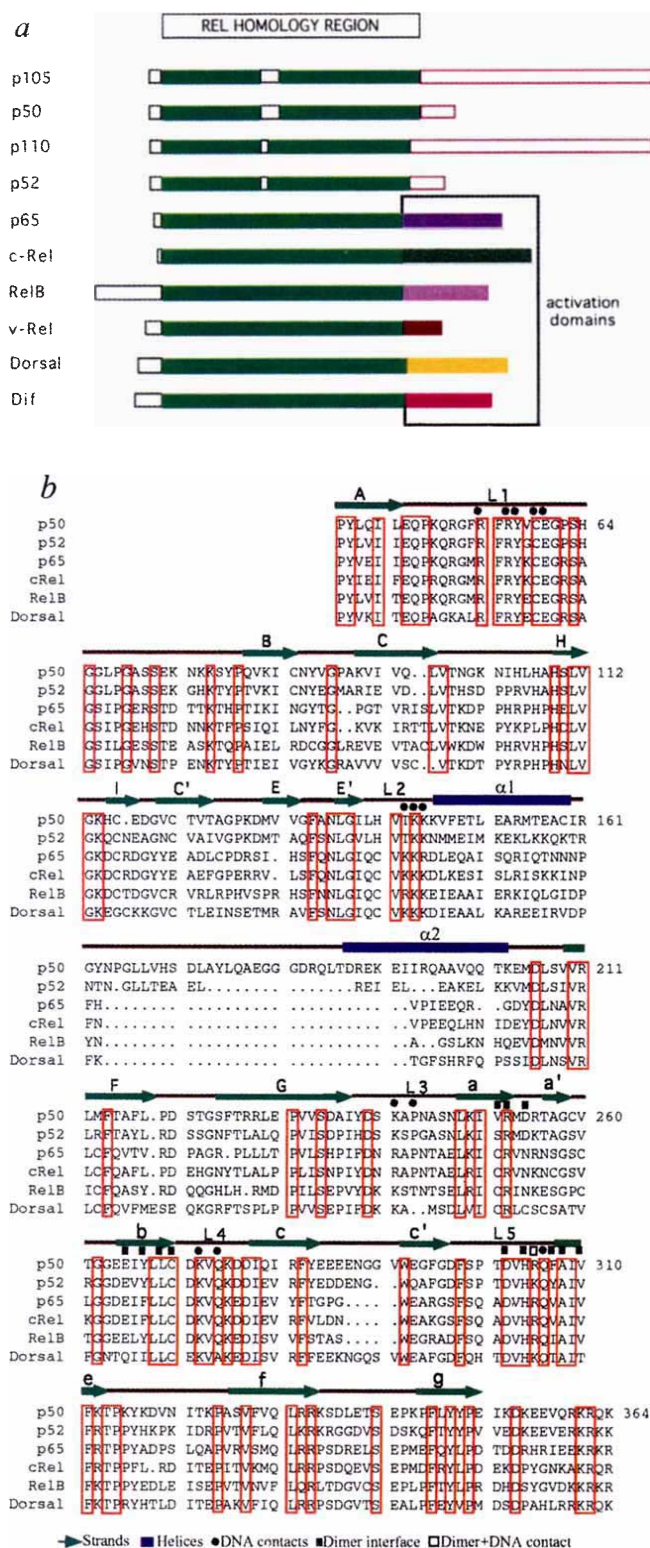
A truncated version of mouse p50 containing residues 39–364 was expressed in soluble form in *Escherichia coli* and purified to homogeneity. This was the smallest RHR construct that would bind to both a κ B site with wild-type affinity and to I- κ B. We chose a 10-base-pair idealized palindromic κ B motif derived from the consensus sequence (Fig. 1d) in order to avoid any crystallographic packing disorder that might be associated with an asymmetrical sequence bound by a symmetrical dimer. The length of our κ B duplex is the minimum required for specific binding by p50. The 5'-overhanging T in our construct (Fig. 1c) was chosen by trial and error to produce the best crystal form. The structure was determined at 2.8 Å by multiple isomorphous replacement using iodinated oligonucleotides and single wavelength anomalous scattering data from selenomethionone-containing (Se-Met) crystals. The Bijvoet differences of the anomalous diffraction from the Se-Met derivative measured at the X4A beamline (NSLS) were critical in obtaining initial phases. The current model was refined to an *R*-factor of 0.23 (free *R*-factor=0.34) for data from 6–2.3 Å with excellent stereochemistry. Small segments of three loops not contacting DNA (residues 177–182, 220–223, 286–289, indicated by asterisks in Fig. 2b) and the C-terminal 14 amino acids in each subunit had uninterpretable electron density. Details of the structure solution and the refinement results are shown in Table 1 and representative electron density in Fig. 4.

General features of the structure

The three-dimensional structure of the p50 homodimer/ κ B complex resembles a butterfly with the protein domains as wings connected to a cylindrical body of DNA (Fig. 2a, b). The most distinctive feature of the specific DNA–protein interface in this complex is that it is formed by well defined loops that connect the β -strands. There is no helical structure in the recognition surface as often seen in specific protein–DNA interactions, nor do the β -strands interact with the major groove, as is seen in the DNA complexes of MetJ, Arc and Mnt¹⁹. Loops are used in class II restriction endonucleases and p53²⁰ to make sequence-specific interactions with DNA targets, although the number and size of the loops are smaller.

The p50 subunit consists of two distinct domains connected by a 10-residue linker (L3, 238–247). The linker adopts a well

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defined conformation in the complex, forming a turn at Pro 243 that lies at the centre of the major groove of the DNA with Lys 241 reaching into the groove to make a specific contact. Five loops from each subunit (including the linker) combine to form an extensive specific contact surface with the functional groups of the major groove and the bordering sugar-phosphate backbone, a distribution that is consistent with the observation that the entire RHR is responsible for κ B binding. The protein-DNA interface buries 3,550 Å² of solvent-accessible area. Except for a slight bend in the very centre (−10° roll) and a slight unwinding (about 11 bp per turn) the κ B segment in the complex does not

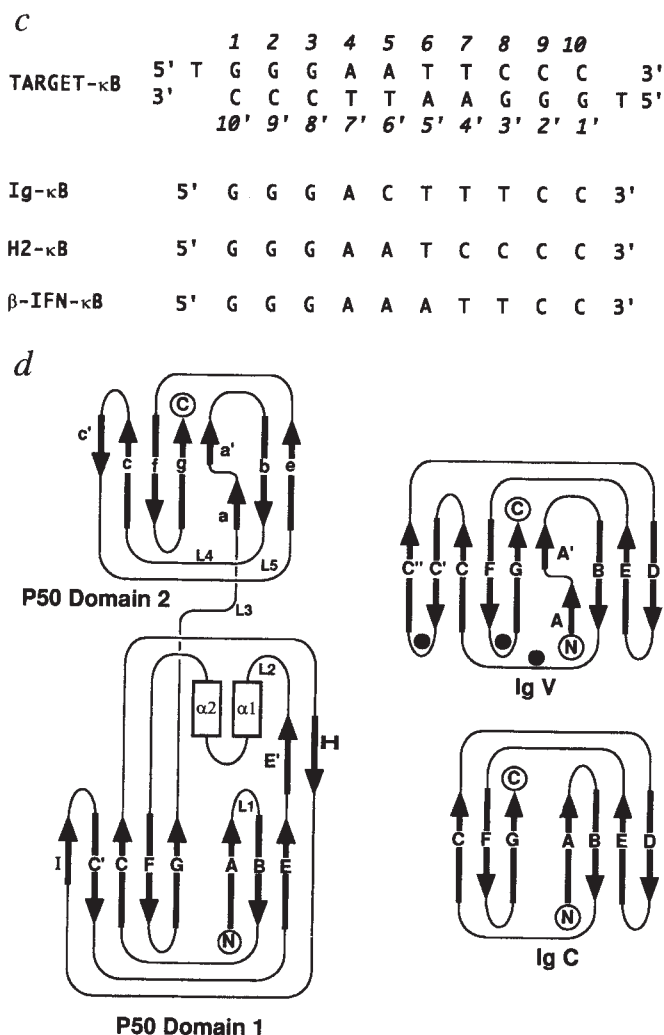


FIG. 1 Sequence and secondary structural features of Rel/NF- κ B homology region (RHR). **a**, Diagram of the sequence motifs in the Rel/NF- κ B family. p50 and p52 are proteolysis products of p105 and p110, respectively. Proteolysis removes the C-terminal I κ B-like domain. Green bars represent region of high homology among the family members (RHR). Different colour bars beyond the RHR represent non-homologous activation domains. **b**, Sequences of Rel homology regions of mouse p50, p50B, p65, c-Rel, RelB and the *Drosophila* protein Dorsal. Green arrows above denote residues in β -strands and purple bars α -helix in p50 RHR. L1 to L5 are loops that contact DNA. Residues in p50 that contact DNA (filled circle), or contribute to the dimer interface (filled square) or both DNA and dimerization contact (empty square) are shown. Boxes enclose identical residues in all six RHRs. **c**, Homodimeric p50 binding site used in this crystal structure and κ B elements found in the intronic enhancer element of κ light chain (Ig- κ B), human β -interferon gene enhancer κ B site (β -IFN- κ B), and major histocompatibility class 1 κ B element (H2- κ B). **d**, Folding patterns for the NF- κ B-p50 RHR (left) and canonical Ig light chain (right). ●, Antigen binding loops in the IgV domain.

deviate significantly from canonical B-form DNA, nor is there any mechanism evident that would severely distort the DNA.

Both p50 domains share a β -sandwich core folding pattern typical of proteins in the immunoglobulin superfamily. Domain 1 includes residues 39–240 and the ABE/GFCC' β -sandwich found in immunoglobulin constant domains. A 77-residue segment is inserted between strands E and F that includes an extension to strand E and an antiparallel pair of helices that pack tightly against the β -sandwich. This segment, which includes 30 residues present in p50 that are absent in the other RHR sequences, approaches the DNA from the minor groove side so

TABLE 1 Summary of crystallographic analysis

(a) Phasing statistics									
Resolution limit (Å)	18.7	10.3	7.1	5.6	4.4	3.7	3.2	2.8	All
Phasing power*†									
Se-Met	0.42	0.62	0.89	0.82	0.53	0.43	0.43	0.42	0.50
Se-Met (anomalous)	0.36	0.47	0.62	0.62	0.61	0.51	0.41	0.29	0.45
I1‡	0.42	0.40	1.79	2.19	1.51	1.37	1.39	1.35	1.41
I2§	3.9	1.38	1.66	1.85	1.05	0.89	0.89	0.87	1.00
I3	0.65	1.04	1.18	1.36	0.96	0.83	0.79	0.84	0.91
Mean figure of merit	0.23	0.43	0.60	0.64	0.55	0.49	0.46	0.30	0.45
(b) Data collection statistics									
	Source¶			Resolution limit (Å)			<i>R</i> _{sym} *		Complete (%)
Native	CHESS A1			2.3			6.8		96
Se-Met	NSLS X4A			2.8			5.8		94
I1	NSLS X12C			3.0			4.6		94
I2	CHESS A1			2.8			8.6		75
I3	CHESS A1			2.8			8.1		48
(c) Refinement results									
	Resolution (Å)			<i>R</i> -factor			Free <i>R</i> -factor		No. of reflections
Data with <i>F</i> > 3σ	6–2.3			0.23			0.34		28,020
All data	6–2.3			0.25			0.37		30,826
r.m.s. deviations	bond lengths 0.01 Å			bond angles 2.0°			improper dihedral angles 2.0°		

Truncated p50 (residues 39–364) was overproduced in a T7 polymerase-based expression system (Novagen) in *E. coli* and purified to homogeneity in three chromatographic steps (S-sepharose (Pharmacia), pH 7.5; Mono-S, pH 8.1). Sample was eluted from the Mono-S column using a 100–400 mM salt gradient; a superdex-200 gel filtration (Pharmacia) was used in the final step. Oligonucleotides were synthesized by the phosphoramidite method and purified by anion exchange chromatography on a MonoQ column at pH 11.8 at room temperature. After concentrating to 2 mM, DNA was annealed and mixed in a 10% molar excess over purified p50 dimers. Monoclinic crystals ($C2$, $a = 84.2$, $b = 132.1$, $c = 80.1$ Å, $\beta = 93.1^\circ$) grew in three days at 18 °C ($0.1 \times 0.1 \times 0.5$ mm) from 4 μ l hanging drops containing 8 mg ml⁻¹ complex, 25 mM HEPES, pH 7.5, 5 mM dithiothreitol, 50 mM ammonium chloride, 5 mM CaCl₂ 0.05 mM spermine, 0.05% (w:v) β -octyl-glucoside, 4% polyethylene glycol 4000 when equilibrated over a reservoir containing double these concentrations, except complex. Before data collection, crystals were washed for 1 min in a cryosolvent that included all reservoir components plus an additional 4% PEG 4000 and 25% glycerol, mounted in nylon loops, and flash frozen in liquid propane. Frozen crystals were stored in liquid nitrogen until needed. These crystals contained one full complex in the asymmetric unit and diffracted well to 2.8 Å at 100K on laboratory sources and to at least 2.3 Å using synchrotron radiation. CHESS A1 data were measured using a CCD detector. To maximize the anomalous signal from selenium, NSLS X4A data were measured and processed using Fuji image plates at $\lambda = 0.9791$ Å. NSLS X12C data were measured using a MAR image plate detector. All data were processed using programs DENZO and SCALEPACK (Z. Otwinowski). Selenium sites were located from the anomalous difference Patterson map and iodines were located from isomorphous difference Fourier using the Se-Met-derived phases. Heavy-atom parameters were refined and phases computed with MLPHARE (Z. Otwinowski). The initial map was improved by non-crystallographic symmetry (NCS) averaging, histogram matching, and solvent flattening with the program DPHASE (G. Van Duyne) and the resulting map allowed us easily to fit the DNA, the α -helices, most of the β -strands, and all of the loops in the protein–DNA interface. The interpretation was completed after iterative cycles of partial model refinement and experimental phase combination. The known coordinates of all five internal methionine sulphur positions in each subunit from the Se-Met derivative provided an internal check to the sequence assignment. Model building and adjustments were done using the program O³⁶ and the structure was refined with X-PLOR³⁷ using both simulated annealing and conjugate gradients minimization with data from 6–2.3 Å. The current model includes 596 amino acids out of 652 for the p50 dimer, 22 nucleotides, and 317 water molecules with refined temperature factors <70 Å² at unit occupancy. A 3D–1D profile analysis¹⁸ for the protein gave a total score of 290 for the dimer, or 145 for a 311 amino-acid subunit, values consistent with the scores of well refined protein structures from the protein data bank. Three out of the 596 ordered residues (His 64, Tyr 87 and Thr 261) have ϕ , ψ angles in disallowed regions.

* Phasing power = $\sum |F_H| / \sum ||F_{H,\text{obs}}| - |F_{H,\text{calc}}||$.

† Anomalous phasing power = $\sum |F_H^*| / \sum ||AD_{\text{obs}}| - |AD_{\text{calc}}||$.

‡ I1: 5'-(TGGGAAU'U'CCC), where U' = 5-iododeoxyU.

§ I2: 5'-(TGGGAAU'TCCC).

|| I3: 5'-(TGGGAATU'CCC).

¶ CHESS A1, Cornell High Energy Synchrotron Source beamline A1; NSLS X4A and NSLS X12C, National Synchrotron Light Source beamlines X4A and X12C.

* $R_{\text{sym}} = \sum |I_h - \langle I_h \rangle| / \sum I_h$, where $\langle I_h \rangle$ is the average intensity over symmetry equivalents.

as to clamp p50 on to the DNA target with highly polarized hydrogen bonds between the central phosphates and the side chains of Lys 144 and Lys 145, and the peptide NH of Lys 144. Because the β -sheets of this N-terminal domain flare away from their symmetrical counterparts, this domain does not contribute to the dimer interface.

Domain 2 includes residues 248–350 and also has a fold similar to the immunoglobulin BF set (C. Chothia, A. Lesk and A. Murzin, personal communication). This domain is responsible for protein dimerization and also plays an important role in DNA binding through loops L4 (272–279) and L5 (304–307). A tight dimer interface is formed by 12 side chains from strands B, D and E interacting symmetrically with the corresponding residues in the opposing subunit, burying a total of 1,500 Å² of solvent-accessible surface (Fig. 3). The centre of the interface is hydrophobic, formed by seven highly conserved side chains (Val 251, Tyr 267, Leu 269, His 304, Phe 307, Ala 308 and Val 310) and is flanked by salt bridges formed by Arg 252 with Asp 254 and Glu 265. The methylene groups of Arg 305 are

packed against the Phe 307 side chain of the opposing subunit as it reaches to interact with the phosphate backbone of DNA. The local protein diad is coincident with that of the DNA. The key residues that form the dimer interface are strongly conserved among Rel family members and the structure reported here should provide a framework for understanding dimerization within this family.

Protein–DNA interactions

Backbone contacts. Figure 3a–c shows that 12 of the 18 phosphates of the target are contacted by at least one hydrogen bond. The six free phosphates are at the ends of the target. The absence of ion pairs at the end of the κ B element is compensated electrostatically by a pair of arginines from each subunit contacting a pair of flanking Gs at each end of the κ B element. All together there are 16 positively charged residues in the interface, of which all but four lysine residues (Lys 144 and Lys 145 from each subunit) are supplied by loops in the major groove. Two additional phosphate contacts are provided by the backbone NH of

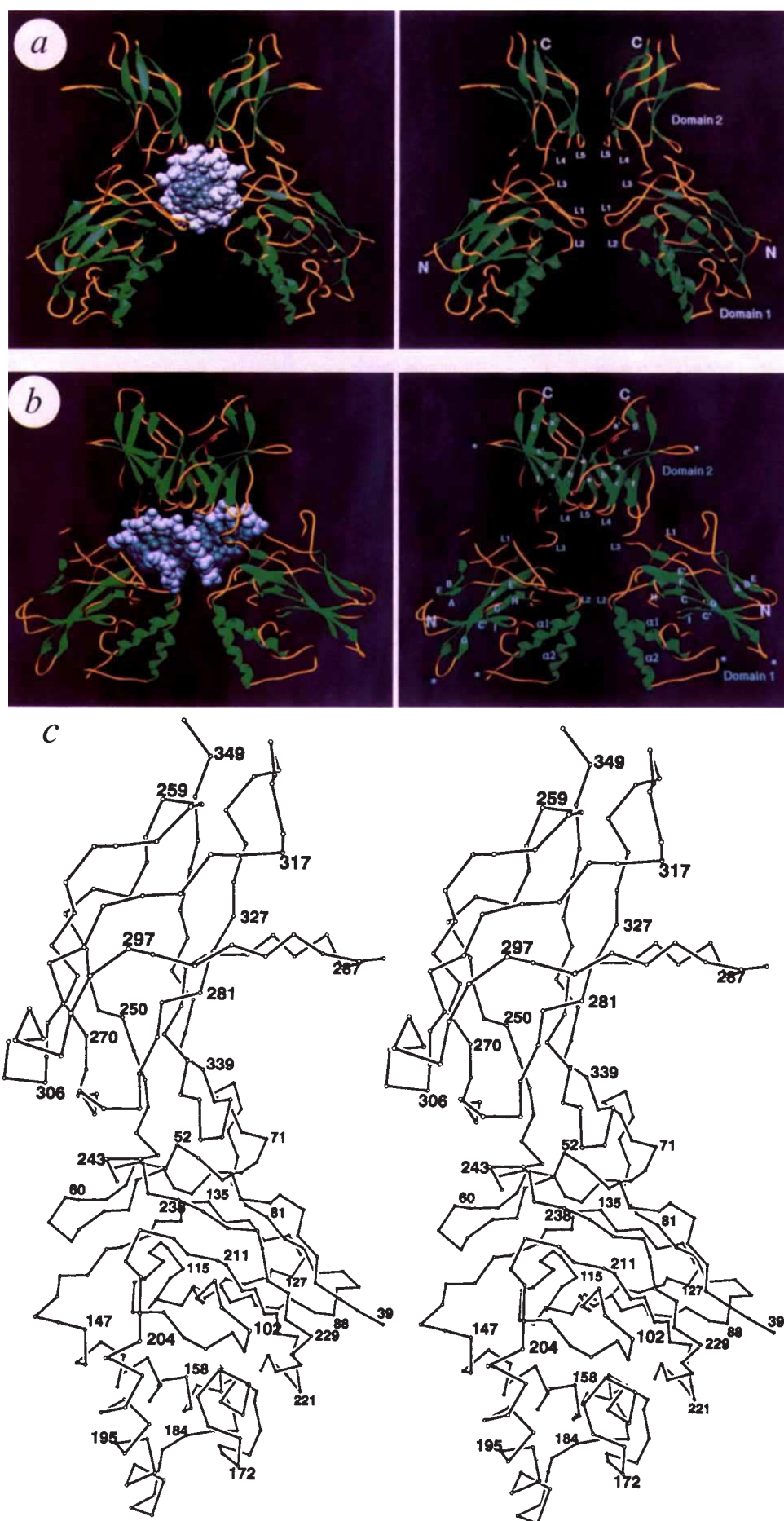
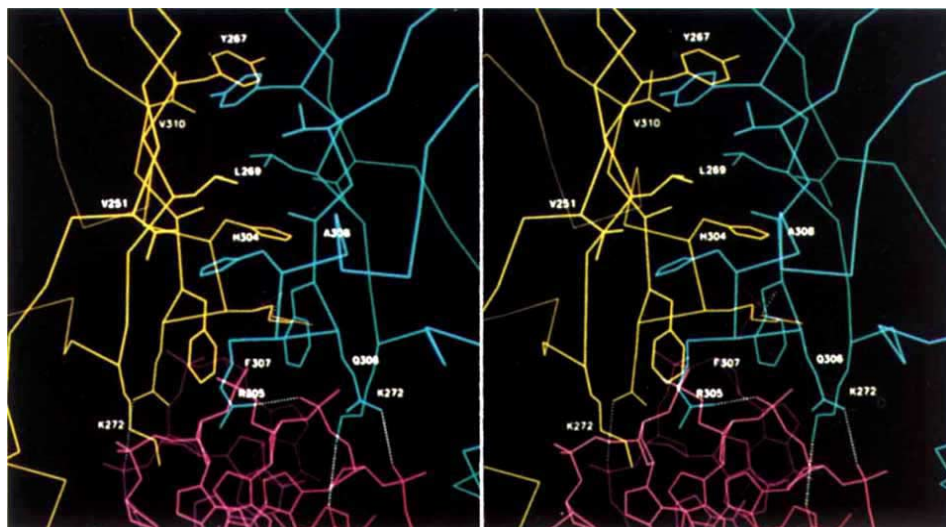
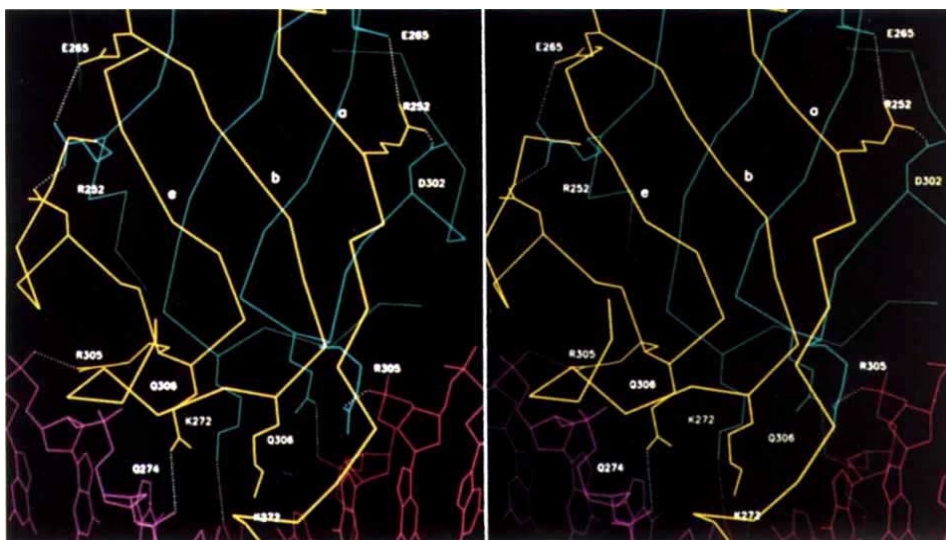


FIG. 2 Structure of homodimeric NF- κ B p50 on an idealized κ B element. **a**, Ribbon drawings of entire complex viewed down the DNA-helical axis (left); right, the same view with the van der Waals space-filling model of the κ B element removed and the DNA contacting loops labelled. **b**, The figure in **a** rotated 40° around the vertical dyad, with the secondary structure elements labelled and the locations of disordered segments indicated by asterisks. **c**, Stereo pair of a p50 subunit C α -trace. **d**, Stereo pair of the dimer interface core with C α s and interacting side chains shown. **e**, Figure in **d** rotated 90° around the local dyad to display polar contacts on the outer surface that stabilize the dimer.

d*e*

Lys 144, which, like the two lysine residues noted above, arises near the N terminus of the $\alpha 1$ helix.

Specificity determinants. The stereochemistry of specific NF- κ B-DNA interactions in this idealized homodimeric p50 complex must be cautiously interpreted in the context of a diverse range of homo- and heterodimers that interact with a wide range of target sites. Whereas *in vitro* target site-selection experiments shows the target site used here is nearly ideal, p50 homodimers and heterodimers bind to various variants of this sequence with physiological affinity and specificity²¹. As mentioned above, the dimer interface could affect the orientation of loops and side chains so that various pairings of RHRs may result in preferences for different target sequences. In this context we note that DNA-binding motifs of most transcription factors that are composed of secondary structure elements present an explicit binding interface, whereas loops, such as the DNA-binding loops of *EcoRV*²² and those used by the RHR to bind DNA, may be more flexible and adjust to different sequences, different binding partners and possibly to neighbouring transcription factors.

There is another unusual feature of the NF- κ B-Rel interaction that undermines a precise understanding of specificity.

Unlike many bipartite protein-DNA complexes where the interaction is restricted to a pair of isolated target half sites, Fig. 3a-c shows that the specific interaction occurs over a 10-bp continuous interface in which the specifying base contacts contributed by one subunit interleave with those of the opposing subunit.

Most of the contacts shown in Figs 1b and 3a have been confirmed by mutational analysis²³⁻²⁸. There is only one site of mutational change shown to affect DNA binding that is not clearly accounted for in our structure²³⁻²⁸. His 64 appears positioned to make DNA contacts to a helical extension of the sugar-phosphate backbone beyond the 10-bp κ B element used in the cocrystal. In this structure the unpaired terminal nucleotides are distorted by packing contacts. As with Tyr 57, Cys 59 and Thr 143, His 64 may make significant van der Waals contact in addition to a hydrogen bond to an extended target. The contacts of Cys 59 to the sugar-phosphate backbone explain the sensitivity of DNA binding to oxidation at this site²⁴, and the presence of Tyr 57 in the middle of the interface formed by L1 accounts for its photochemical crosslinking to DNA targets²⁶.

The interactions of the highly conserved loop between strands A and B in domain 1 (L1) with the κ B site explain the require-

ment for two GC base pairs at each end of nearly all κ B sites. Figure 3b–d shows that the key to this discrimination is an array of interactions nucleated by Glu 60, which not only contributes its backbone carbonyl and a side chain oxygen to direct interactions with the 4-amine of the two cytosines, but also buttresses the flexible side chains of Arg 54 and Arg 56 in their interactions with the two guanines. In addition, the stereochemical impact of Glu 60 and the optimal conformation of loop L1 are reinforced through a hydrogen bond between the backbone amide of Glu 60 and the carbonyl of Tyr 57 which contacts a phosphate through its phenolic hydroxyl. Interestingly, the T-cell-specific transcription factor NF-AT, which shares 20% sequence identity with the RHR, also uses this highly conserved glutamate²⁹.

Role of the linker and dimer interface in κ B recognition. The p50 and p65 subunits of NF- κ B form heterodimers that are at least as stable as p50 homodimers and favour asymmetrical κ B sites. Excluding the variable insert (residues 164–193), the sequence identity exhibited by p50 and p65 in the RHR indicates a similar fold and presumably a similar overall architecture for the p65 homodimer and p50–p65 heterodimer complexed with their target DNA. There are, however, notable exceptions that may lead to small but functionally significant structural adjustments in the dimer interface causing the DNA-contacting residues in loops 3, 4 and 5 to match better the heterodimer-specific κ B. For example, Phe 307 forms part of the interface by contacting its counterpart in the opposing subunit (Fig. 2d), providing a van der Waals surface that guides the direction taken by the side chains of Gln 306, Arg 305 and Lys 272. Interestingly, two of these side chains contact the central four base pairs that distinguish most κ B sites. The Phe 307→Val substitution in p65 may therefore alter the mode of interactions of these side chains with the target by providing an alternative surface to orientate the functional groups.

Coupled with the use of fixed water molecules to mediate interactions with functional groups on the DNA bases, this potential for alternative structures may contribute to a mechanism for Rel dimers to distinguish their targets. Indeed, the plasticity of the centre region of the interface is reflected in the lack of symmetry exhibited by the interactions of Lys 241 from the linker segment, and Lys 272 and Arg 305 from the dimerization region with the symmetrical target site.

Müller *et al.*³⁹ have independently determined the crystal structure of an NF- κ B p50 homodimer bound to a similar target which contains an 'extra' base at the very centre (5'-...GGGGAATCCCC...3'). Although the dimerization domains are superimposable, the N-terminal domains are oriented slightly differently, permitting similar specific contacts closer to the diad axis in their structure.

Interaction with I- κ B

Two mutational changes in Dorsal at residues corresponding to Thr 256 and Ala 257 in p50 disrupt interaction with the dorsal-specific inhibitory protein Cactus (M. Ptashne, personal communication). These residues are located just above (see Fig. 2) the dimer interface, facing away from the DNA interaction surface of p50. *In vitro* mutagenesis has also shown that some I- κ B proteins bind to the nuclear localization signal (NLS) and contact the L1 loop³⁰. In this structure we do not see the C-terminal 14 residues, including the NLS (residues 360–364). This portion of the RHR is highly charged and not likely to be an integral part of the domain's structure. The last ordered residue (Glu 350) in the complex is, however, close to residues 256 and 257. The linker region connecting the two p50 domains may be very flexible when uncomplexed and domain 1 could assume a different orientation with respect to the dimerization domain. Given the hinge-like appearance of the linker segment, it seems possible that the NLS, L1 and the region surrounding residues 256 and 257 could combine to form a composite surface to interact with an I- κ B.

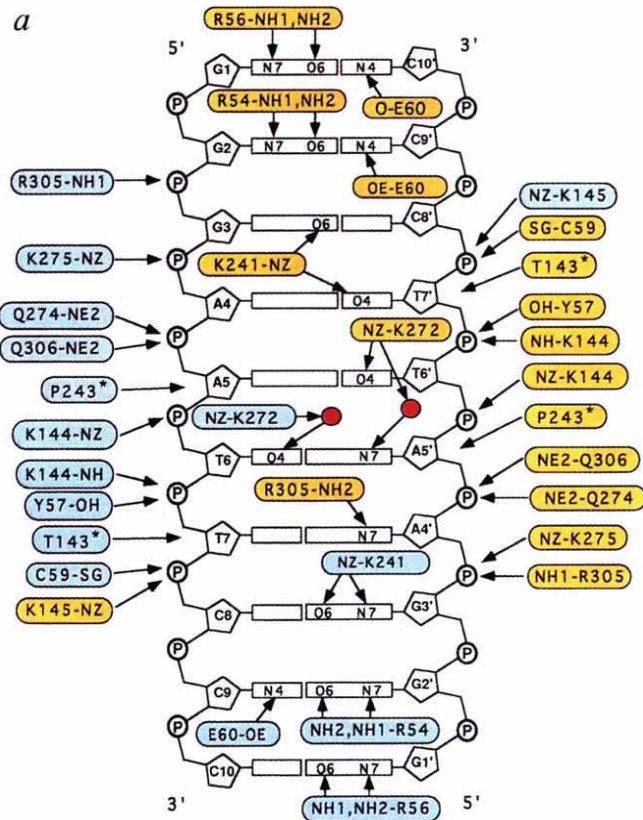


FIG. 3 Protein–DNA interactions in homodimeric NF- κ B p50 complex with idealized κ B element. **a**, A schematic representation of all interactions seen in the crystal structure. Yellow and blue distinguish the two subunits. Arrows pointing to polar functional group are hydrogen-bonds; *, non-polar van der Waals contact; red dots, water-mediated hydrogen bonds. **b**, Stereo pair α trace (blue) of both p50 subunits and complete skeletal model of κ B (red and purple) displaying side chains (yellow) that make hydrogen bonds to DNA. **c**, Stereo pair magnification of the central region in **b** showing hydrogen bonds (white dots). **d**, Stereo pair showing the specific interactions between p50 and the G1:C10', G2:C9' basepairs of the κ B site.

Interaction with other transcription factors

NF- κ B/Rel proteins function with other transcription factors such as helix–loop–helix (HLH), high mobility group (HMG), and bZIP proteins³. Protection and modification studies have implicated the minor groove of the specific κ B element (PRDII) as the binding surface for HMG I(Y) which has been shown to co-occupy the PRDII element *in vitro* and coactivate from PRDII *in vivo*³¹. Access by HMG I(Y) at the centre of the PRDII element in the presence of NF- κ B p50/p65 is required for such function. There are no contacts in the minor groove at the centre of the homodimeric p50 complex to prevent binding by HMG factors. Figure 4 shows that the helical segment between strands E and F of domain 1 is near the presumed HMG contact site. This sequence is significantly different and shorter in p65 so that any restrictions imposed by the nearby helices may be partially relieved in p50–p65 heterodimers.

Similarity to the immunoglobulin fold

At first glance the folding patterns of the RHR domains strongly resemble those of immunoglobulins³², in that domain 1 has nine antiparallel β -strands, like IgV, and domain 2 has seven antiparallel strands, like IgC. There are clear differences, however. Figure 1d shows that the immunoglobulin-like core of domain 1

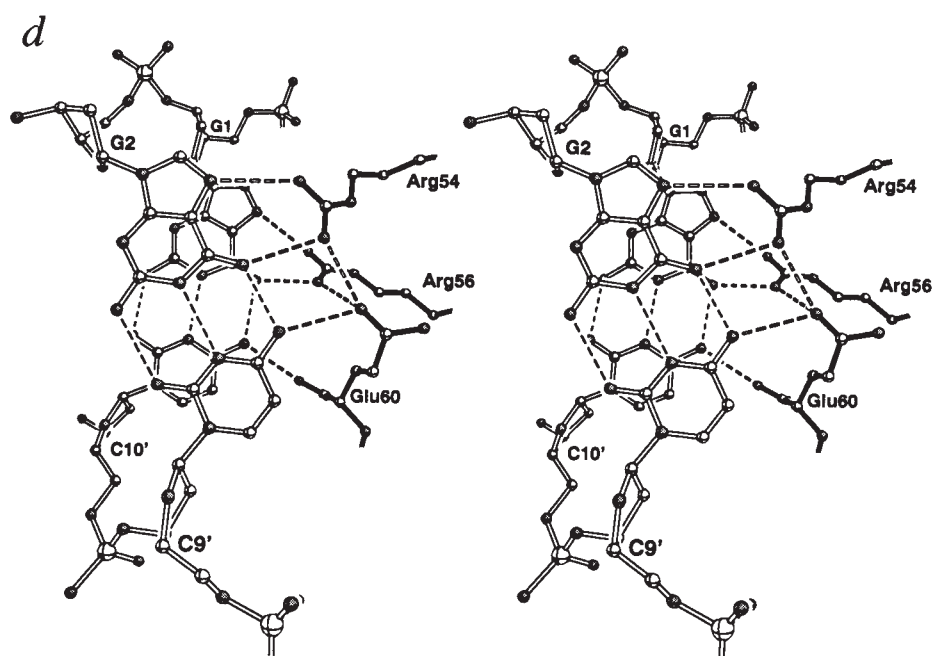
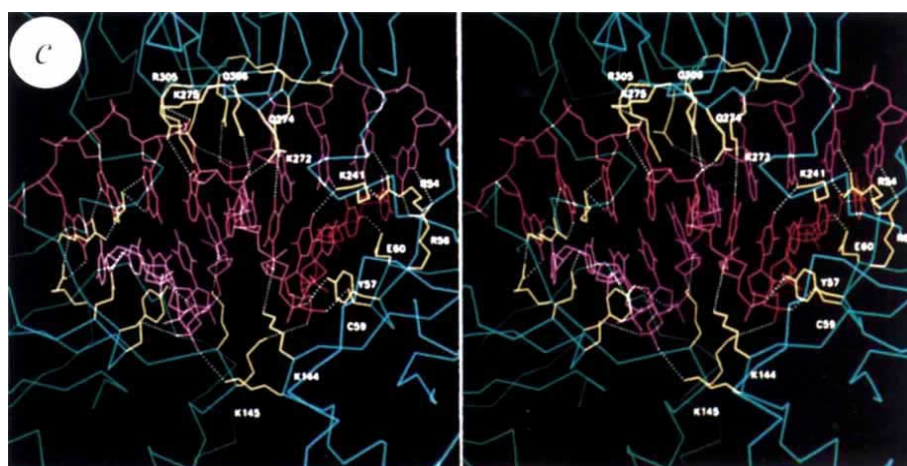
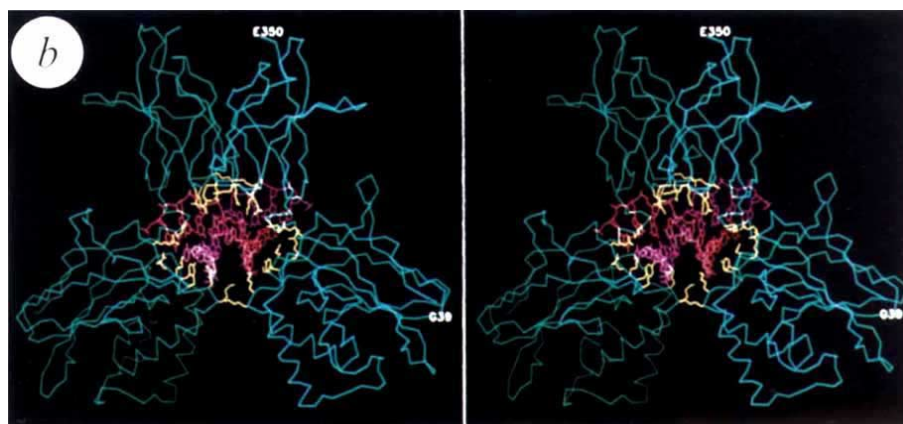
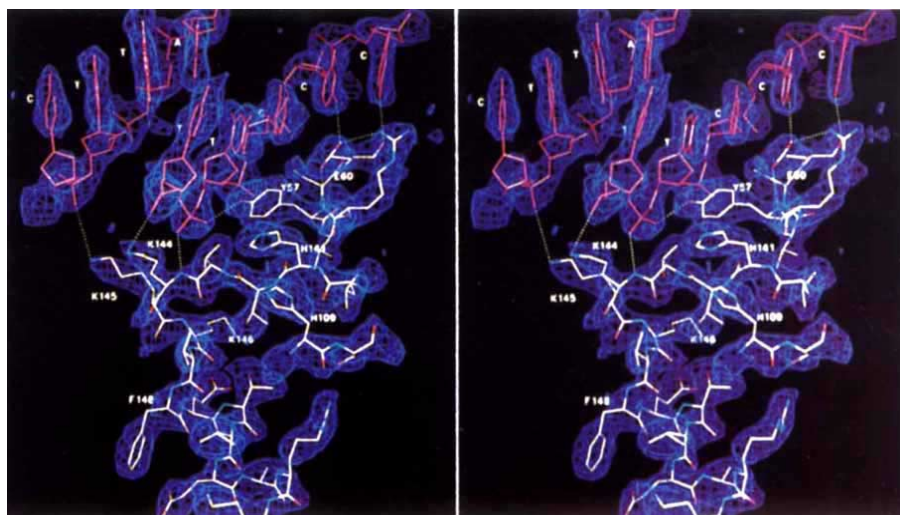


FIG. 4 Stereo view of representative electron density ($2F_o - F_c$, 1.5σ) and underlying skeletal model showing residues near the N terminus of $\alpha 1$ interacting with the phosphate backbone, including the main chain NH of Lys 144 and the side chains of Lys 144 and Lys 145 near the minor groove. Also shown are contacting residues from the large and well buttressed loop 1 (L1).



deviates from IgV in the connectivity of the flanking two strands. Similarly the connectivity of domain 2 differs from IgC in the position and orientation of the flanking two strands. The folding pattern most closely related to domain 1 (excluding the helical insert) is exhibited by a member of the actinoxanthin family³³. It is of interest to the evolutionary considerations discussed here that these domains are from antimicrobial agents that are a primitive defence mechanism. The most closely related folding pattern to domain 2 is the BF set of the immunoglobulin superfamily, exhibited in fungal galactose oxidase and bacterial cyclodextrin glucosyl transferase (C. Chothia and A. Lesk, personal communication). The evolutionary linkage to the RHR, if any, is obscure. The most distinctive departure from the actinoxanthin folding pattern occurs between strands E and F where a 56 amino-acid segment is inserted. A 30 amino-acid segment within the 56 amino-acid insert is unique to p50 and the remaining 26 amino acids are poorly conserved among the Rel family proteins. This segment is flanked by a pair of helices, one of which ($\alpha 1$) is described above in connection with phosphate contacts that straddle the minor groove at the centre of the κB element.

The most striking difference between the structure-function relationship in NF- κB and the immunoglobulins is the manner in which they bind their respective targets. Whereas the DNA target is bound between the NF- κB domains, antigens are bound towards the end of the IgV domain. Of the loops used by each subunit to bind the κB element, only one loop corresponds to a loop in IgV that binds antigen. In this context we note that the extracellular portion of hGHR, composed of two C2 set domains, binds its macromolecular ligand, growth hormone,

between the identical subunits and through an interface provided mostly by loops from both domains³⁴, that is, in a way similar to that in which p50 binds to its ligand, the κB element.

Members of the immunoglobulin superfamily play important roles in vertebrate immune and neural systems. In both systems, immunoglobulin domain-containing proteins are generally extracellular, either circulatory or anchored to membranes, where they mediate specific interactions with other cell surface molecules. Immunoglobulin-related molecules often exist as dimers, and the binding between immunoglobulin domains can be either homophilic, for example as in antibodies, or heterophilic as in the T-cell receptor. Also, cell adhesion can occur through homophilic or heterophilic binding. It has been generally accepted that immunoglobulin domains evolved from an ancestral primordial domain through gene duplication and divergence. It would appear now that the immunoglobulin superfamily can be extended on the basis of structural similarity to the NF- κB /Rel family of transcription factors. Moreover, like the extracellular immunoglobulin proteins, function and regulation of NF- κB /Rel are mediated in part by the formation of homo- and heterodimers. The emergence of extracellular/cell surface immunoglobulin proteins in insects, including the neural adhesion molecules fascilin II and III, neuroglian, and the secreted protein amalgan, is paralleled in the NF- κB /Rel family by the existence of the transcription factors Dorsal and Dif⁶. Dif coordinates the insect innate 'immune' response by controlling the expression of antibacterial peptides in response to infection^{13,35}. How far into the evolutionary past this connection of structure and function persists will be an intriguing subject for future study. □

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Structure of the NF- κ B p50 homodimer bound to DNA

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The structure of a large fragment of the p50 subunit of the human transcription factor NF- κ B, bound as a homodimer to DNA, reveals that the Rel-homology region has two β -barrel domains that grip DNA in the major groove. Both domains contact the DNA backbone. The amino-terminal specificity domain contains a recognition loop that interacts with DNA bases; the carboxy-terminal dimerization domain bears the site of I- κ B interaction. The folds of these domains are related to immunoglobulin-like modules. The amino-terminal domain also resembles the core domain of p53.

THE mammalian transcription factor NF- κ B, first isolated as the protein specifically bound by a site in the κ immunoglobulin light-chain enhancer¹, regulates expression of a large number of genes involved in the response to infection and stress^{2,3}. Some viruses, including human immunodeficiency virus, subvert it to activate transcription of their own genes. NF- κ B is a heterodimer of subunits known as p50 and p65 (RelA), both of which exhibit homology to the product of the *rel* oncogene^{4,7}. The *Drosophila* morphogen Dorsal is a non-mammalian homologue⁸. The similarity among these proteins, known as the Rel-homology region, extends over about 280 amino-acid residues. It contains determinants of dimerization and of specific DNA binding. Mutational dissection has not, however, revealed a smaller subfragment that binds DNA on its own. Rel family members can form hetero- and homodimers with distinct specificities in various combinations^{9,10}. The p50 homodimer is present in the nucleus of some cells; one important target appears to be a site in the major histocompatibility complex (MHC) class I gene enhancer^{11,12}.

Import into the nucleus is a critical step for the regulation of NF- κ B activity^{2,3}. NF- κ B is present in the cytoplasm of many cells, bound to an inhibitory protein, I- κ B¹³. The nuclear localization signal (NLS) in NF- κ B, which is just C-terminal to the Rel homology region, and a set of ankyrin repeats in I- κ B, are jointly required for the interaction¹⁴⁻¹⁶. Masking of the NLS by I- κ B results in cytoplasmic retention. Inducers (such as lipopolysaccharide, phorbol ester or viruses) of NF- κ B activation appear to trigger dissociation or degradation of I- κ B^{2,3}.

NF- κ B p50 (about 435 residues) is the N-terminal part of a precursor, known as p105, which contains a set of ankyrin repeats in its C-terminal half⁴. Proteolytic removal of the C-terminal region leads to nuclear import of p50 homodimers, which themselves bind to specific DNA sites^{14,17,18}. Expression in *Escherichia coli* of the p50 part of human p105 yields soluble and active protein which can be trimmed proteolytically to a stable fragment that ends precisely at the NLS¹⁹. This dimeric fragment, NF- κ B p50(2-366), binds specifically to sites recognized by NF- κ B. We have crystallized it in complex with a synthetic oligodeoxynucleotide bearing the sequence of an MHC class I enhancer site²⁰. We describe here the structure of this complex, determined at 2.6 Å resolution using X-ray crystal-

lography (see Table 1 and Fig. 1). A related structure is reported in the accompanying paper²¹.

The p50 subunit

The Rel homology region of p50 consists of two distinct domains (Fig. 2). The larger, N-terminal domain contains residues that contact DNA base pairs; the smaller, C-terminal domain forms the dimer interface as well as an extensive set of DNA phosphate interactions. The short segment connecting the two domains must serve as a hinge, because the dimer grips the 11-base-pair (bp) DNA duplex like a set of jaws and needs to open in order to bind or dissociate (Fig. 2a).

The N-terminal domain contains residues 43 to 244; 2 to 42 are present in our crystals, but they are disordered. Proline 43, the first residue for which we see clear electron density, is also the beginning of the Rel-homology region as conventionally designated. Residues 160 to 205 (roughly) are not part of the Rel consensus. They form a compact, partly α -helical structure within the domain itself.

The core of the N-terminal domain is a nine-strand, anti-parallel β -barrel, from which emanate several important loops (Fig. 2). The assignment of strands and their arrangement into sheets are shown in Fig. 3. Strands ABGD form one sheet; EFCHI the other. The G strand has a discontinuity, and we have denoted the two parts G and G'. The fold is similar to the core domain of the p53 tumour-suppressor gene product, although a number of the strands are of different length and the loops are quite distinct²². Except for strands D and E, the β -barrel in this domain superposes well in three dimensions on that of p53 (see legend to Fig. 3). We have not, however, been able to detect sequence similarity, even after a structural alignment. The common fold of these two proteins is related to the immunoglobulin fold; strands A, B, C, F, G, H and I in p50 correspond to A, B, C, C', E, F and G in an immunoglobulin variable domain (see legend to Fig. 3).

The only α -helices are in the large GH loop, which contains residues unique to p50. Sequence alignment (Fig. 3a) suggests that p65 and other Rel homology proteins lacking this insert will contain the proximal parts of α A and α B, with a short connection (Fig. 2c). Thus, the unique structures in p50 are in the distal parts of helices α A and α B, and the connecting region with a short α -helical segment. These structures pack against the concave outer surface of the EFCHI sheet.

TABLE 1 Summary of the crystallographic analysis

	Data statistics				MIR analysis			
	Resolution (Å)	Reflections measured/unique	R_{sym}^* (%)	Completeness (%)	Isomorphous† difference (%)	Phasing power‡ acentric/centric	Figure of merit acentric/centric	f'/f'' § (electrons)
Crystal set I: $a=b=136.3$ Å, $c=57.2$ Å								
Native I	3.0	41,840/10,412	6.7	92.3	—	—	0.41/0.68	—
Di-iodo-U	3.4	31,677/7,150	6.9	91.8	14.4	0.7/0.6	—	—
Di-iodo-C	3.4	24,569/7,314	8.9	93.9	21.7	1.3/1.0	—	—
Mono-iodo-C	3.8	17,876/5,551	5.6	98.4	17.5	0.9/0.7	—	—
Crystal set II: $a=b=138.4$ Å, $c=57.2$ Å								
						MIR and MAD analysis		
Native II	3.1	65,730/10,545	6.5	99.2	7.0	0.6/0.4	0.51/0.64	—
Se-1 ($\lambda_1=0.9810$ Å)	3.4	25,537/7,715	5.2	95.5	—	—	—	−8.83/3.21
Se-2 ($\lambda_2=0.9801$ Å)	3.4	26,166/7,727	5.8	95.7	2.9	0.4/0.3	—	−7.48/4.21
Se-3 ($\lambda_3=0.9000$ Å)	3.4	27,668/7,804	5.5	96.6	7.5	0.3/0.2	—	−1.60/3.30
Di-iodo-U	3.4	30,686/7,872	6.3	96.9	18.0	0.8/0.5	—	—
Di-iodo-C	3.8	8,385/4,775	5.8	84.7	22.7	1.3/0.8	—	—
Di-I-U-I-C	4.0	15,803/3,846	6.1	78.5	14.0	1.2/0.9	—	—
Di-I-U-di-I-C	4.0	18,085/4,781	5.5	97.0	16.5	1.2/0.7	—	—
Crystal Set III: $a=b=137.0$ Å, $c=57.0$ Å								
						Refinement statistics		
Native III	2.6	86,474/16,434	4.5	95.8	Resolution: 6.0–2.6 Å	Total number of atoms: 2,836		
						Water molecules: 47		
						R.m.s. bond length (Å): 0.010		
						R.m.s. bond angle (degree): 1.8		
						Crystallographic R -factor (%): 23.3 (for 13,094 reflections)		
						Free R -factor (%): 32.7 (for 1,450 reflections)		

Summary of the structure determination: NF- κ B p50(2-366) was overproduced in *E. coli* and purified as described¹⁹. The selenomethionine-substituted protein was expressed using the same plasmid in a methionine auxotroph⁴¹. A C62A mutant yielded slightly larger crystals, isomorphous to wild type, and the mutant was therefore used in the structure determination. Oligonucleotides and their iodo-derivatives were synthesized on a Milligen DNA synthesizer and purified by reverse-phase HPLC. Iodinated DNA was protected from light during synthesis, purification and crystallization. A single oligonucleotide strand was used in the crystallization which had the sequence 5'-AGAT*GGGGAATCCC*C*T*AGA-3' (bases marked with asterisks were iodinated in some of the derivatives). Crystals were obtained using hanging-drop vapour diffusion by mixing 1–2 μ l of 0.18 mM protein dimer and 0.22 mM DNA duplex in 0.1 M NaCl, 10 mM HEPES, 1 mM DTT with 1–2 μ l reservoir solution containing 50 mM sodium acetate, pH 4.7, 50 mM MgCl_2 , 1 mM DTT, 2 mM spermine and 1–4% PEG 3000 as precipitant. Crystals grew within 2 days typically to a size of $250 \times 250 \times 400$ μm . The space group is $P4_12_12_1$. The crystals were harvested into 10% PEG 8000, 50 mM sodium acetate, pH 4.7 and 50 mM MgCl_2 . To allow data collection at -160 °C, crystals were then transferred to a glucose-containing harvest buffer. Starting at 2% (w/v), the glucose concentration was increased stepwise until it reached a final concentration of 25% (w/v). We observed a variation of the cell dimensions among different sets of frozen crystals. The a and b axes varied between 136.3 and 138.4 Å, and the c axis from 57.0 to 57.2 Å. A combination of multiple wavelength anomalous diffraction (MAD), multiple isomorphous replacement (MIR), and molecular averaging⁴⁵ of maps from crystals with slightly different cell constants was used to solve the phase problem. Data for crystal set I and for the di-iodo-C derivative of set II were collected on a Siemens area detector with an Elliot-GX13 X-ray source and processed with XDS⁴². The remaining data were collected at the NSLS Brookhaven National Laboratory on phosphor image plates and integrated using DENZO (Z. Otwinowski and W. Minor, personal communication). Data from derivatives di-I-U-I-C and di-I-C-di-I-U were collected at the HHMI beamline X4A; the MAD data, the native datasets II and III, and derivative di-iodo-U were collected on beamline X25. For the MAD data collection, a selenomethionine crystal was aligned with its four-fold axis along the camera axis and perpendicular to the beam, permitting the simultaneous recording of Bijvoet pair reflections. Data were collected at three different wavelengths: the inflection point ($\lambda=0.9810$ Å), absorption maximum ($\lambda=0.9801$ Å) and one remote wavelength ($\lambda=0.9000$ Å) of the selenium K-edge; the Se-absorption edge was determined by measuring X-ray fluorescence at 90 degrees from the crystal. Data at the inflection point and absorption maximum were recorded alternatively in batches of 12 degrees; data at the remote wavelength were collected afterwards, with 48 degrees of data being recorded at each wavelength. The rotation range per exposure was 1.0 degree. All data were collected from one crystal. The last exposures showed radiation damage even at -160 °C. Iodine positions in crystal set I were initially located by difference Patterson synthesis. Refinement of the heavy-atom parameters with MLPHARE (CCP4 Program suite) yielded the first electron density map at 3.5 Å resolution. The MAD data from 30 to 3.5 Å resolution were scaled using the local scaling program NEWLSC (A. Friedman, personal communication). Scaling the Bijvoet pairs of acentric reflections at all 3 wavelengths resulted in an R_{sym} of 4.5% compared to an R_{sym} of 3.7% for centric reflections. Values of f' and f'' were subsequently refined using the program MADRBE (A. Friedman), a modified version of MADLSQ⁴³. Five of the seven selenium atoms were located in an anomalous difference Fourier synthesis using MIR phase from iodo-U and iodo-C substituted DNA derivatives. The two remaining Se-Met residues are in the disordered amino-terminal segment. Best phases for crystal set II were obtained by using native II, Se-1, Se-2, Se-3 and the iodine derivative in a 'conventional' MIR/anomalous refinement with Se-1, as the reference set⁴⁴. Electron density maps at 3.5 Å resolution resulting from crystal sets I and II were improved by averaging between the two slightly different crystal forms⁴⁵. The observed non-isomorphism, a 1.5% change of cell parameters along the a and b axes, proved sufficient for a dramatic improvement in the electron density after averaging. An initial model built in this map included about 70% of the DNA and 60% of the protein. Phases from this model were combined⁴⁵ with the averaged phases from crystal sets I and II and also used to phase native data from crystal set III. Iterative averaging among all three sets yielded a good map at 3.2 Å resolution (Fig. 1a), which was used to complete the structure. Model building was carried out using program O (ref. 46); program XPLOR⁴⁷ was used for crystallographic refinement. The final model (Fig. 1) includes all of residues 43–352, with the exception of 223–228, which are in a loop too disordered to build. Other regions with high thermal parameters include residues 76–80, 181–192 and 321–326. The model has excellent geometry and a crystallographic R -factor of 23.3% (free R -factor, 32.7%) for all data with spacings between 6.0 and 2.6 Å. The DNA in the crystal is aligned along axis c . The structure shows that the DNA forms an 11-bp duplex with a central A · A mismatch. The 5' ends from strands in adjacent cells interact with each other across a crystallographic dyad through A · T and G · A base pairs. The 3' ends also form an A · T pair between bases 16 and 19.

* $R_{\text{sym}} = \sum_i |I(i, h) - \langle I(h) \rangle| / \sum_i I(i, h)$, where $\langle I(h) \rangle$ is the mean of the i observations of reflection h .

† Isomorphous difference is $\sum_h |F_{\text{PH}} - F_{\text{P}}| / \sum_h F_{\text{PH}}$, where F_{P} and F_{PH} are the derivative and native structure factor amplitudes respectively. In crystal set II, locally scaled data of Se-1 to 3.5 Å resolution were used as a reference data set.

‡ Phasing power, mean value of heavy-atom structure-factor amplitudes divided by the lack-of-closure.

§ f'/f'' represents the real and imaginary components of the anomalous scattering factor of selenium; f'/f'' of Se-3 were fixed during the MADRBE refinement.

|| The free R -factor⁴⁸ was calculated with 10% of the data.

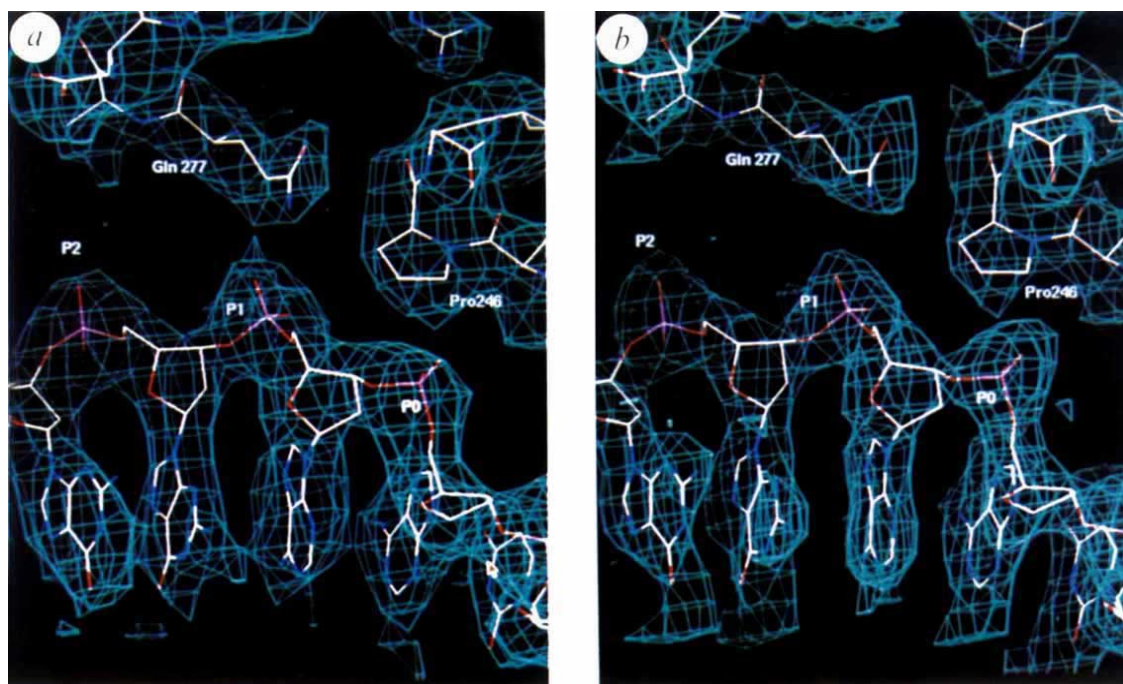


FIG. 1 Electron density around the phosphate of adenine 1: note how proline 246, in the linker between domains, contacts the deoxyribose between P0 and P1. *a*, Triple-averaged electron density map at 3.2 Å

resolution (Table 1). The contours are at 1.3 σ . *b*, $2F_o - F_c$ map of the refined model at 2.6 Å resolution. The contours are at 1.1 σ .

The C-terminal domain includes residues from 250 to the end of the expressed fragment. The last residue that is sufficiently well ordered to be included in our model is 352. Only nine residues (all but one highly polar) intervene between this point and the NLS. The domain is a β -barrel with the fold of an immunoglobulin-like module. In particular, it corresponds to an 'h-type' immunoglobulin fold, as recently defined²³. Other examples include domains in bacterial and fungal enzymes, such as cellulase²⁴. We use lower-case letters, beginning with 'a', to designate the strands. The abed sheet faces the dyad. The ab, cc' and ef loops decorate the distal tip of the domain, together with the C terminus. The ab loops of 2-fold related subunits create a groove at the dyad. They are supported by the ef loop and by the C-terminal strand. The cc' loop projects some distance from the core of the β -barrel. Its β -hairpin conformation may be stabilized in part by a crystal contact. This projection, which contains four sequential glutamate residues, is truncated in p65 and c-Rel (Fig. 3*a*).

The linker between the two domains folds back as it passes from Lys 244 to Asn 250. It is not well anchored to either domain by hydrogen bonds or by extensive hydrophobic contacts, and it thus has the characteristics required for a flexible hinge. Indeed, the interface between the N-terminal and C-terminal domains is not tightly packed, and most of the interdomain contacts are polar. We imagine that when the p50 dimer dissociates from DNA, the linker is somewhat extended and the two domains are rather loosely tethered.

The DNA site

The DNA in our crystals contains the palindromic sequence of the NF- κ B MHC class I enhancer, a preferred site for the p50 homodimer (Fig. 4). We used a single synthetic strand, which forms an 11-bp duplex having an A · A mismatch at its centre. Binding studies show that this mismatch has little effect on affinity (C. Larson, personal communication). Successful crystallization with non-complementary ends was observed by chance, and the sequence of the DNA ends was then optimized. There are four nucleotides to either side of the self-complemen-

tary part, and these make additional base pairs between fragments bound to different p50 dimers as described in the legend to Table 1. In Figs 2, 4 and 5, we illustrate only the central 11 bp, because the conformation of the ends is determined by interactions unique to the crystal lattice.

The 11-bp duplex has several unusual features (Figs 2 and 4*c*). The overall twist is about 10.7 bp per turn, and there is a very deep major groove. The DNA axis bends toward the dimerization domains by 15° at the junction between the central AAT sequence and the GGGG half-sites. The minor groove is quite narrow across the dyad (about 7 Å from O4' to O4'). The conformation of the GGGG segments resembles the 'extended groove' B-form structures recently analysed²⁵. Because the continuous duplex is only 11 bp long, the grooves are open at either end, and it is not possible to make strong conclusions about the actual overall bend or about the effects of likely backbone contacts outside the 11-bp region.

DNA recognition

The DNA is in contact with both domains of p50. The protein dimer lies over the major groove, and it wraps around it from either side of the interface between C-terminal domains. The 2-fold axis that relates the p50 subunits coincides with the central dyad of the DNA (and with a crystallographic dyad). The N-terminal domains flare outward from their positions over the GGGG half-sites. Part of the AB loop projects into the groove, contacting base pairs. We term this the 'recognition loop', and we describe its interactions in detail below. It is different from DNA recognition motifs previously described. The importance of its sequence for DNA recognition has been demonstrated²⁶. An additional major-groove contact comes from the last residue in the N-terminal domain (Lys 244).

Phosphate contacts from parts of the C-terminal domains appear to anchor the dimer and to position it over the centre of the binding site. The residues that participate in hydrogen bonds and salt bridges to phosphates 1 and 2 are found in the bc and de loops. They include Lys 275, Gln 277, Arg 308 and Gln 309. Pro 246, in the interdomain linker, contacts deoxyribose 1 (see

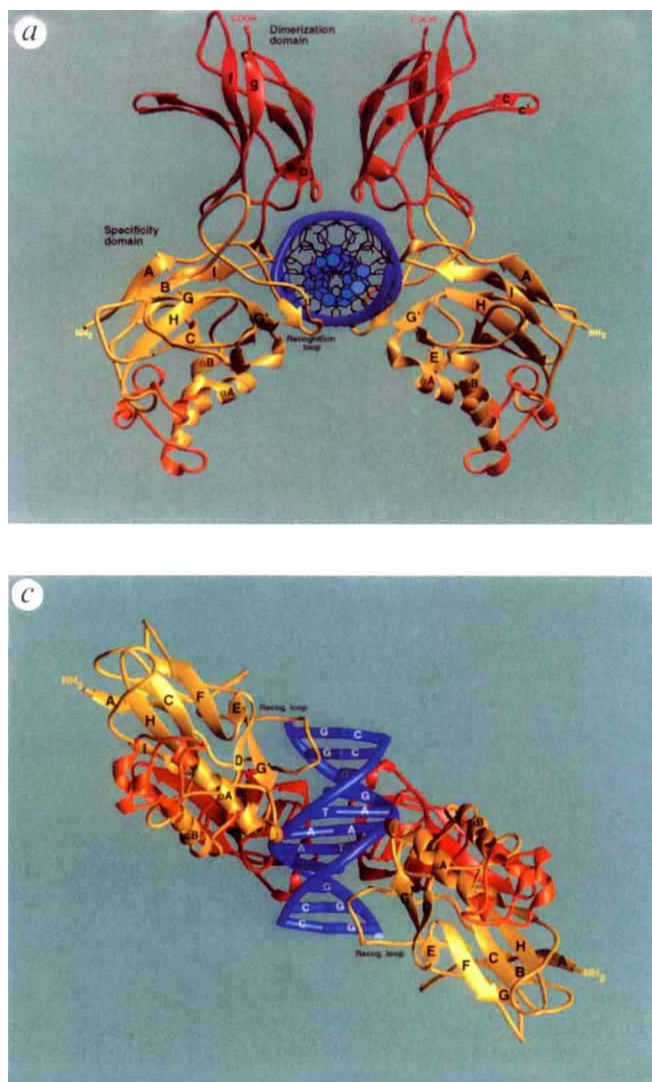


Fig. 1). These residues are almost entirely conserved in the Rel family.

The N-terminal domain is also anchored to the sugar phosphate backbone. Phosphate -1 receives three hydrogen bonds, from the main-chain $-NH$ of Lys 147 and from the side chains of Tyr 60 and His 144. The side chain of Lys 147 extends toward phosphate 0. The ring of Tyr 60 lies against ribose -1 . There are likely to be additional contacts with phosphates outside the 11-bp site, that is, on the edge of the major groove just distal to the GGGG sequence. Because the conformation of the DNA in our crystals is not continuous beyond the 11bp, however, we can only infer such contacts from the position of residues that might participate. In particular, it is likely that the second part of the AB loop, which contains three lysine residues, arches over the sugar-phosphate backbone and forms, together with the recognition loop, a C-shaped clamp across the DNA strand.

A network of hydrogen bonds links the residues of the recognition loop with the outer three guanines, the inner two cystosines, and phosphate -1 (Fig. 4). At the centre of the loop-DNA interface are the side chains of two arginines and a glutamic acid. These three residues, which are conserved throughout the Rel family, form a defined recognition motif for two sequential guanines. The guanidinium groups of arginines 57 and 59 are roughly coplanar with guanines 3 and 4, respectively, and donate hydrogen bonds to O6 and N7. The bidentate hydrogen-bonding contacts, predicted for arginine-guanine pairs²⁷, have been seen

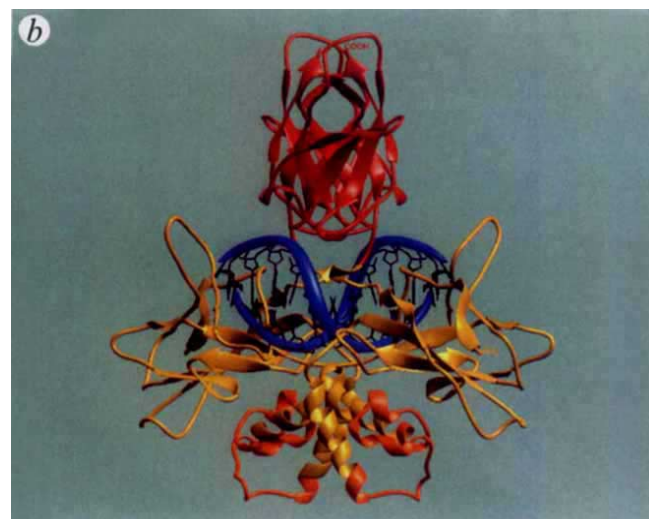


FIG. 2 Overall view of the NF- κ B p50 dimer bound to DNA. Depicted are residues 43 to 352 of both subunits and the central 11 bp of the DNA. The colour coding is yellow for the N-terminal domain, orange for residues 164 to 191 (the 'insert' in p50 with respect to other Rel homologues), red for the C-terminal domain and blue for the DNA. Identical colour coding is used in other figures. Labels A-I and labels a-g mark the strands of the N-terminal and the C-terminal domain, respectively. For clarity, residues 223-228 in loop HI, which is disordered in our crystals, have been modelled in the sketch. All model figures were generated using the program Ribbons³⁶. a, View along the DNA, with the dyad vertical. b, View perpendicular to the direction of the DNA. The orientation of the dyad is maintained. c, View along the dyad of the complex from below.

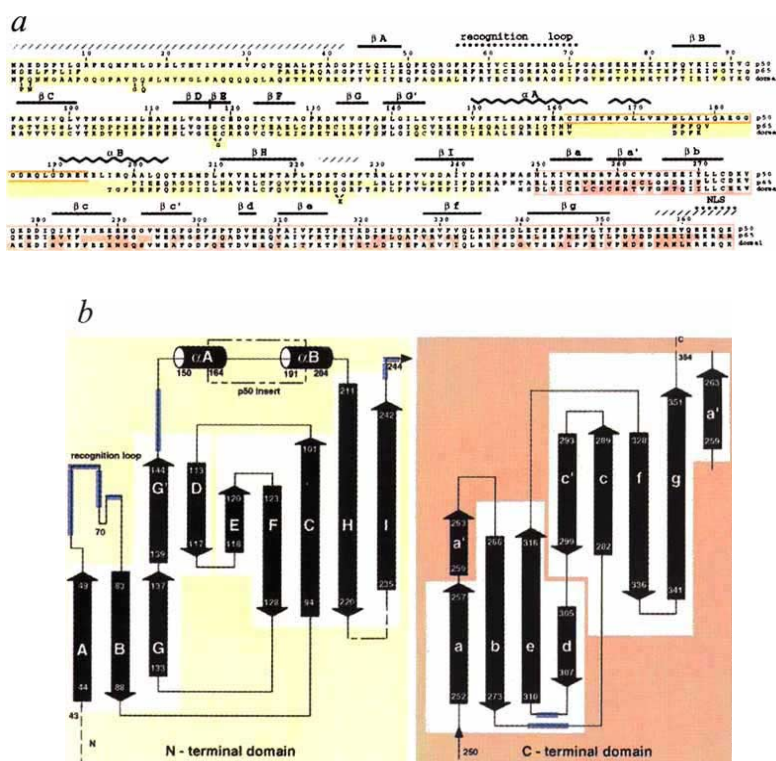
in other structures^{28,29}. Glu 63 supports the two arginyl side chains by accepting hydrogen bonds from the NH_2 s. It also accepts hydrogen bonds from the N4 atoms of cytosines 2 and 3. To one side of this conserved Arg-Arg-Glu unit lies the side chain of His 67, which donates a hydrogen bond to Gua 5. Its imidazole ring forms a continuous stack with the guanidinium groups of arginines 57 and 59. To the other side of Arg 57 is the side chain of Tyr 60, which donates a hydrogen bond to phosphate -1 . Tyr 60 can be photo-crosslinked very efficiently to a bromo-dU at the position of Cyt -2 ³⁰: its phenol ring would indeed contact the Br atom of the modified base. The side chain of cysteine 62, mutated to alanine in our protein, would lie near phosphate -2 . Modification of this cysteine with bulky reagents interferes with DNA binding²⁶. Cysteine residues with similar properties lie adjacent to phosphates in DNA complexes of Fos and Jun³¹.

The only residue not in the recognition loop to make contact with bases is Lys 244, which lies at the boundary of the N-terminal domain and the linker. It donates hydrogen bonds to O6 of Gua 2 and to O4 of Thy -1 . This lysine can be crosslinked to a modified Ade 1; it is the only side chain in the vicinity of the adenine base³².

Site specificity

Do the interactions just described account for observed p50 specificities? G · C base pairs at positions 3 and 4 would appear

FIG. 3 a, The amino-acid sequence of residues 1–366 of human p50 (ref. 5) aligned with those of the corresponding parts of p65 (ref. 6) and Dorsal⁸. Numbering refers to human p50. Conservations are shown as grey boxes around the letters. Secondary structure assignments are indicated by lines (β -strands) and zig-zags (α -helices) above the sequence. Hatched bars above the sequence show regions too disordered to include in our model. b, Folding diagrams for the two domains of p50. Strands are shown as arrows and helices as barrels. Several single turns of 3_{10} - or α -helix are not included, nor is one short α -helix in the insert region. The first and last residues of secondary structural elements are numbered; hydrogen bonding was used as a criterion for the assignment. The coloured backgrounds in both parts of the figure correspond to the domain colours (yellow and red) in Fig. 2. Blue bars show segments of polypeptide chain involved in DNA contacts. In relating the domains to other proteins, as cited in the text, we have carried out the following superpositions; N-terminal domain on p53 (ref. 22; 43 α -carbon positions in strands A, B, C, F, H and I; r.m.s. difference, 1.6 Å); N-terminal domain on IgV REI (ref. 37; 35 α -carbons in strands A, B, C, F, H and I; r.m.s. difference, 1.14 Å); C-terminal domain on Ig domain of cellulase²⁴ (27 α -carbons in strands b, c, e and f; r.m.s. difference, 2.3 Å); C-terminal domain on fibronectin type III domain of tenascin³⁸ (29 α -carbons in strands b, c, d, e, f, and g; r.m.s. difference, 1.7 Å).



to be most critical (contacts from Arg 57 and Arg 59 supported by Glu 63) with further preference for G · C at position 5 (His 67). The specificity at position 2 is probably less predictable, because the side chain of Lys 244 is relatively unconstrained. The deep major groove and narrow central minor groove in the p50 complex may create further 'indirect' preferences for A · T rather than G · C base pairs at the centre of the site. Selection experiments with p50 yield GGGGATPyCCC as a 10-bp consensus, essentially the sequence in our crystals¹⁸. Similar experiments with p65 yield sites with xGGxxTTTCC as a 10-bp consensus¹⁸. Thus, there is a GG 'core element' in each half site, at positions 3 and 4 (taking the first T as the centre). It is likely that, as in p50, the conserved Arg-Arg-Glu unit recognizes this GG core. The greater tolerance for variation at position 5 may be explained by the presence of an alanine, rather than histidine 67, in the p65 AB loop.

Comparison of our structure with the one also reported in this issue²¹ shows that the half-site spacing is an additional variable in Rel family specificity. The complex described in the accompanying paper²¹ has six base pairs between GG core elements, rather than five as seen here. The difference is accommodated by flexibility in the linker between domains. The possibility of alternative spacings complicates interpretations of selection and binding experiments when the half-site contain continuous runs of a single base, as they do in many sites for NF- κ B.

The DNA binding by hetero- and homodimers of the Rel family can be influenced by other proteins. For example, at the PRDII site of the β -interferon promoter, virus induction requires both NF- κ B and the high-mobility group protein I(Y) (HMGI(Y))³³. HMGI(Y) contacts the AT-rich centre of PRDII in the minor groove, which is open in our structure. Likely contacts on p50 and p65 include the end of the G' strand, α A and α B (Figs 2c and 5b). These have sequences that are not well conserved, and HMGI(Y) could therefore have preferences for particular Rel family members. HMGI(Y) and other proteins

might also affect the favoured half-site spacing, through contacts with the N-terminal domain.

The dimer interface

Unlike the DNA contacts, which come from both domains, the dimer contacts are entirely in the C-terminal domain, as suggested by earlier mutational analyses³⁴. The dimer interface contains a set of hydrophobic side chains from strands b and e at its centre and a set of polar contacts, largely from strands a and d, at its periphery (Fig. 5a). In particular, Tyr 270, Leu 272, Phe 310, Ala 311 and Val 313 from the two subunits interdigitate in a complementary manner at the core of the contact. His 307 interacts with two carbonyl groups across the contact, and it lies near Asp 274. Arg 255 and Asp 305 form salt bridges. Nearly all these residues and some others that participate in the contact are strictly conserved among mammalian Rel family members, and many are conserved in Dorsal. Moreover, the hydrophobic residues projecting toward the interior of the domains in the vicinity of these contacts are also conserved. We therefore anticipate that both the character of the dimerization surface and its shape, as determined by the folded structure of the domain, will permit unrestricted homo- and heterodimeric pairing between Rel proteins, as observed, and we believe that the present structure is a good model for other Rel dimers. Preferences for particular partners are likely to be determined by more subtle differences in the polar contacts at the margins of the interface.

The dimer interface and the DNA backbone contacts from the C-terminal domain lie adjacent to each other in a continuous, conserved surface (Fig. 5a). Thus, the dimerization domains of any two, paired Rel family proteins form a structural unit, which anchors the protein across the major groove at the centre of the binding site. Comparison with the complex described in the accompanying paper²¹ shows that this structural unit can bind with its 2-fold axis coincident either with the dyad through a base pair (as here) or with the dyad between base pairs. Remark-

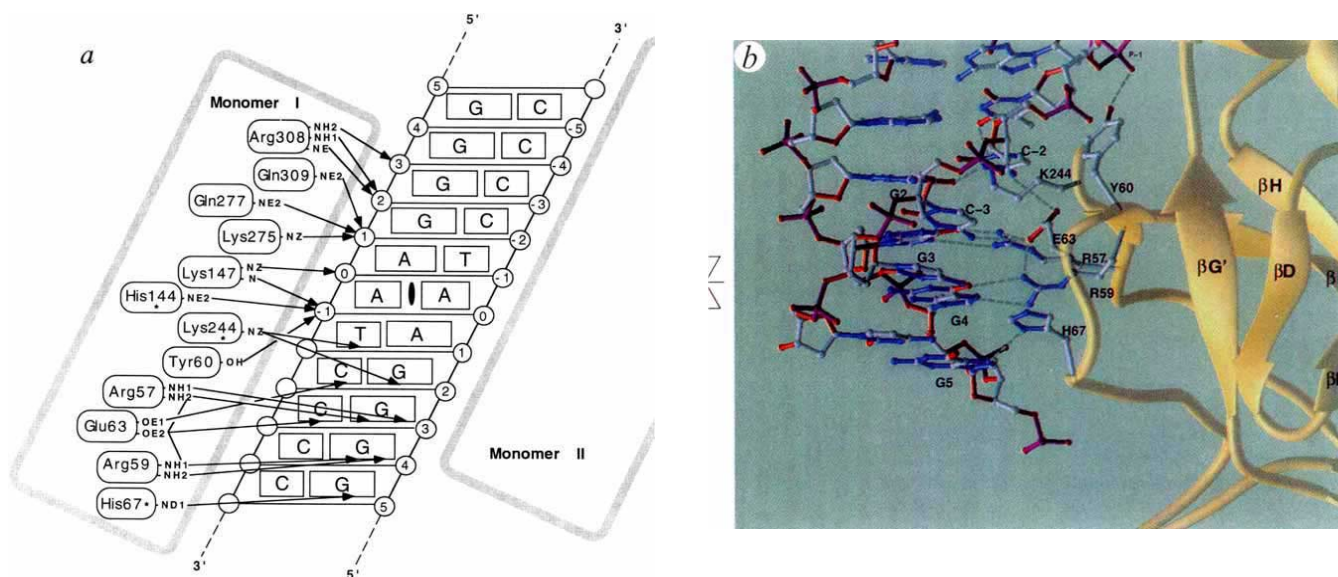


FIG. 4 DNA recognition by NF- κ B p50. *a*, Schematic diagram of the polar interactions between p50 and the central 11 base pairs of the DNA. Interactions between protein and DNA are marked by arrows; interactions within the protein are indicated with lines. The dyad between monomer I and II and the two DNA strands, is shown as $\bullet$. His 67, His 144 and Lys 244 (asterisked) are not conserved between p50 and p65; the corresponding residues in p65 are Ala 43, Cys 120 and Arg 187. *b*, Protein-DNA contacts between one monomer and its DNA half-site. The orientation of this view corresponds to the one in Fig. 2c (lower left). Of the residues depicted, only Lys 244 lies outside the recognition loop. Dashed lines indicate polar interactions. *c*, The 11-bp duplex found in the NF- κ B p50-DNA complex. The helical axis as calculated with the program CURVES³⁹ is shown as a black line. The dyad of the DNA is horizontal in the plane of the sketch, perpendicular to the DNA. The DNA bends towards the dimerization domains, which are located over the major groove on the right-hand side of the DNA. Drawn with MOLSCRIPT⁴⁰.

ably similar sugar-phosphate contacts are made in both cases. Flexion of the linker then allows the N-terminal domain to recognize the half sites in similar ways.

I- κ B binding

Binding of I- κ B to NF- κ B depends on the NLS, which lies just beyond the last ordered residue in our structure. Two mutations in the ab loop of Dorsal have recently been shown to prevent interaction with Cactus, the *Drosophila* I- κ B homologue³⁵. Moreover, a mutation in Cactus suppresses the effects of both Dorsal changes. The ab loop lies adjacent to the C terminus of the dimerization domain, leading to the NLS, and both the loop and the NLS are likely to be contacted by I- κ B (Cactus). The ab loop is directed away from the dimer interface, creating a groove that we propose will receive I- κ B (Fig. 5b). Dimerization is required for I- κ B binding¹⁵. Differences in the sequences of this loop in p50 and p65 imply a somewhat asymmetric site in NF- κ B and potentially different I- κ B specificities for homo- and heterodimers.

Association of NF- κ B with I- κ B generally inhibits DNA binding¹⁵. The I- κ B site implied by the location of the NLS and of the ab loop is separated from DNA by the dimer interface. I- κ B is certainly large enough to extend from the groove between

the dimerization domains to the region of DNA contact, and it could in principle interfere directly. It might also contact the N-terminal domain so as to prevent the inter-domain hinge from adopting the angle required for DNA binding. An attractive third mechanism, which restricts the necessary I- κ B interactions to the region of the ab loop and the NLS, involves an allosteric change in the dimer interface. Because the unit formed by the two C-terminal domains contacts DNA phosphates on both sides of the major groove, any switch in the geometry of the dimer will also affect DNA affinity.

Structural similarities

Both domains of the Rel homology region are related to immunoglobulin modules; the specificity domain resembles the core domain of p53. What do these resemblances mean?

The immunoglobulin fold is very widely distributed, and the Rel homology region illustrates new uses for this versatile unit. The p53 relationship appears to extend beyond geometry to a degree of functional homology. Both domains interact with DNA in an essentially similar way. The same end of the β -barrel faces DNA in both cases (with significant difference in orientation for the best superposition of strands), and the corresponding loops participate in base-pair contacts. The details of

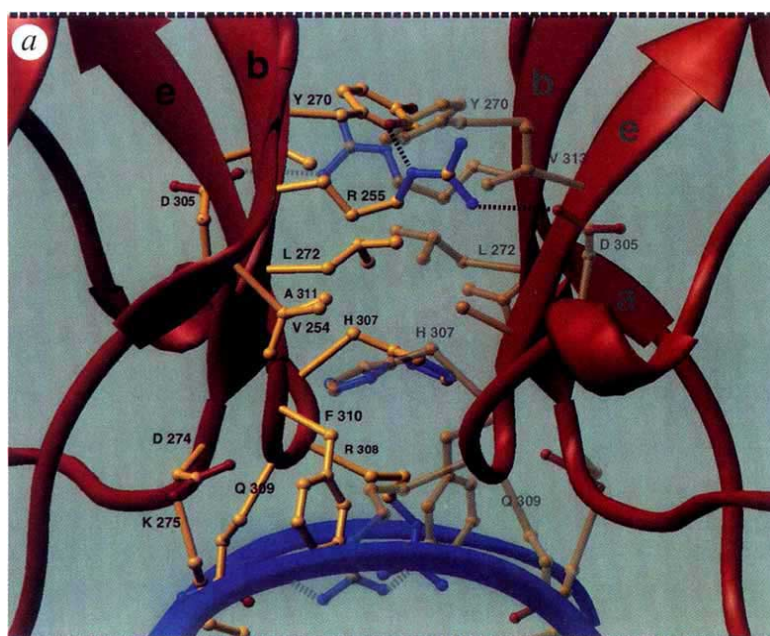
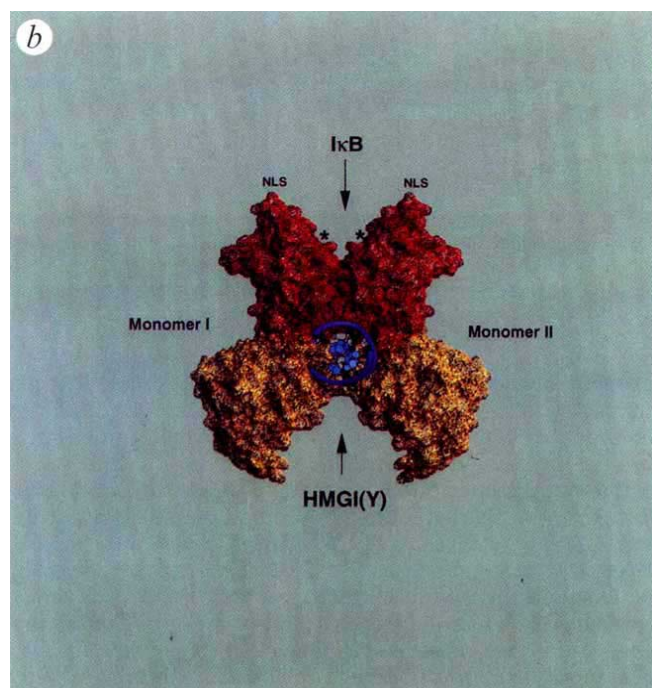


FIG. 5 *a*, The dimer interface, viewed along the DNA axis. Dimer interface and DNA-backbone-contacting residues form a continuous unit. Only the side chains of contact-forming residues are depicted. *b*, Surface representation of the NF- κ B p50 dimer, showing likely regions for interactions with I- κ B and HMGI(Y). Asterisks mark the position of two Dorsal mutants which inhibit binding of the I- κ B homologue Cactus³⁵, and 'NLS' marks the carboxy terminus, leading to the nuclear localization signal. HMGI(Y) interacts with the minor groove at the centre of the PRDII site in the β -interferon promoter³³: it must therefore approach from 'below'.



recognition and of DNA binding differ substantially, but it appears reasonable to say that the two structures belong to a common class of domains that interact with DNA. □

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Production and evolution of light elements in active star-forming regions

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COLLISIONS between cosmic rays (energetic protons and α -particles) and carbon, nitrogen and oxygen in the interstellar medium have been considered¹ to be the main source of lithium, beryllium and boron, through fragmentation of the larger nuclei. But this mechanism is unable to account for the observed Solar System abundances of the isotopes ${}^7\text{Li}$ and ${}^{11}\text{B}$. The recent detection of an excess of γ -rays² in the direction of the star-forming region in the Orion cloud has been interpreted³ as arising from the excitation of carbon and oxygen nuclei ejected from supernovae when they collide with the surrounding gas, which is primarily molecular and atomic hydrogen. Here we investigate the consequences of the two-body interactions of the ejected carbon and oxygen nuclei (and the α -particles ejected with them) with the hydrogen and helium in the surrounding gas, using a model developed previously⁴⁻⁶. We show that these interactions offer a way to make lithium, beryllium and boron that is independent of the abundance of heavy elements in the surrounding medium. Such supernova-driven interactions, combined with the effect of galactic cosmic rays, can explain the observed Solar System abundances of these light elements.

The production of lithium, beryllium and boron in star-forming regions is governed by three factors; the composition and energy spectrum of the fast nuclei, which is related to the progenitors of the supernovae, and the composition of the surrounding interstellar medium. There is no lack of sources of low-energy particles in the Orion cloud (for example, supernovae, stellar winds and flare stars) but a good reason to choose supernovae is that they do not produce an undesirable excess of γ -ray lines in the 1–3 MeV region³. A clue to supernova activity in the recent past is a large interstellar cavity extending from Orion up to Eridanus, formed by supernova plasmas⁷. Here we take as source compositions those of the ejecta of 15, 25, 35 and 60 $M_{\odot}$ stars⁸. These stars are not sufficiently massive to be appreciably affected by mass loss, except in the case of the 60 $M_{\odot}$ star⁹. Subgroup 1c in the Orion OB1 association, which is still embedded in Cloud A, seems to have the appropriate age and location¹⁰ to be the explosion site. The most massive stars presently in this subgroup have masses of $\sim 35 M_{\odot}$. It is therefore plausible that a supernova just above this mass has exploded recently (we urge observers to look for it). Note that in these types of supernovae, carbon and nitrogen are underabundant with respect to O.

For the composition of the surrounding medium we treat two cases, zero metallicity ($Z=0$; all elements heavier than helium are 'metals') to approximate the very early Galaxy, and solar metallicity^{11,12} ($Z=Z_{\odot}$), to enable us to model the evolution of the Galaxy and the Orion cloud, respectively. The models consider $\alpha + \alpha$ fusion and all the reaction channels leading to the production of lithium, beryllium and boron through the breakup of carbon, nitrogen and oxygen nuclei. The energy-dependent cross-sections are taken from an extensive compilation¹³. As the absence of an excess of high-energy γ -rays from π^0 decay in the direction of the Orion region¹⁴ implies a lack of high-energy particles, low-energy threshold reactions are of special importance; for example all reactions including α -particles, which are important because of their low reaction threshold. The latter are

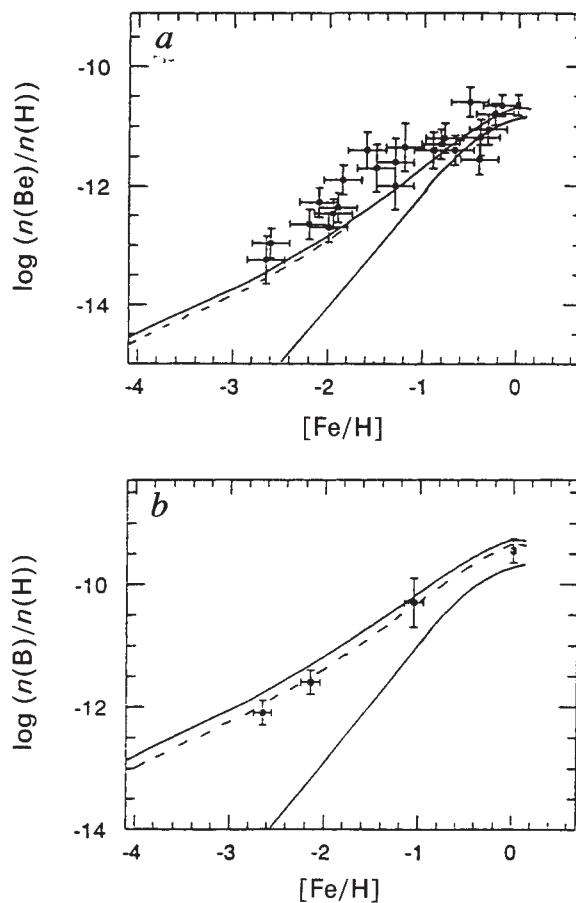


FIG. 1 a, Plot of $\log(\text{Be}/\text{H})$ against $[\text{Fe}/\text{H}]$. Solid line, predictions including the Orion-like and Galactic cosmic ray (GCR) components; data points, observations from Ryan *et al.*²⁶ and Gilmore *et al.*²⁷. b, Plot of $\log(\text{B}/\text{H})$ against $[\text{Fe}/\text{H}]$ as a. Data points, observations from Duncan *et al.*²⁸

favoured because they are abundant in the ejecta of type II supernovae and they are less affected than heavier nuclei by ionization-energy losses in the course of their propagation through the surrounding medium.

This study is limited to considering a simple source spectrum described by a plateau, followed by a power law of index n after a cut-off energy E_c (refs 4, 15). The propagation of the fast particles is treated as usual^{15,16}. Fast particles are confined in the cloud because of their small gyroradius and their fate is dominated by ionization losses (thermalization). The relevant cross-sections are carefully integrated over the equilibrium spectrum to get the production rates. In applying this process to the early Galaxy, we have selected the spectral parameters (E_c , n) to avoid overproducing lithium and beryllium at very low metallicity¹⁷ ($\text{Li}/\text{Be} < 100$), to reproduce the B/Be ratio in extreme population II stars (that is, $\text{B}/\text{Be} \approx 20\text{--}40$)^{18,19} and to get ${}^{11}\text{B}/{}^{10}\text{B} \approx 4$ at solar birth, after mixing with galactic cosmic-ray spallation products¹⁵, along with C to O γ -ray line ratio of the order of 1 at solar metallicity². This requires rather specific

TABLE 1 Abundance ratios

Ratios	60 $M_{\odot}$	GCR
${}^7\text{Li}/{}^6\text{Li}$	5.1	1.4
${}^{11}\text{B}/{}^{10}\text{B}$	6.9	2.5
Li/Be	43.4	12
B/Be	47.0	17

Symbols/abbreviations used; GCR, galactic cosmic rays.

conditions like a $60\text{-}M_{\odot}$ supernova composition, together with, for example, $E_c = 9\text{ MeV}/n$ and $n = 10$. Special conditions of the kind $E_c = 7$ and 9 discussed previously are very sensitive to the rise of cross-sections near threshold. To avoid this problem we take $E_c \approx 10\text{ MeV}/n$. Indeed, only the most massive stars, which have the shortest lifetimes, are expected to explode within giant molecular clouds. The other stars, because of their random motions, should leave the cloud before exploding. (Type Ia supernovae, descending from long-lived low-mass systems are of course supposed to blast in the intercloud medium.)

The isotopic abundance ratios calculated with the above prescription at $Z=0$ and $Z=Z_{\odot}$ are compared to those produced by the standard model, using only galactic cosmic rays (GCR) in Table 1. In the case of the $60\text{-}M_{\odot}$ star the results at $Z=0$ and $Z=Z_{\odot}$ are similar. These isotope production rates normalized to the γ -ray line emission from carbon and oxygen ($3 \times 10^{39}\text{ photons s}^{-1}$) are displayed in Table 2. The predicted γ -ray line emission of Orion at $\sim 450\text{ keV}$ from the prompt de-excitation of ${}^7\text{Li}^*$ and ${}^7\text{Be}^*$ (ref. 20) amounts to only $1.2 \times 10^{37}\text{ photons s}^{-1}$. The $60\text{-}M_{\odot}$ case has ${}^7\text{Li}/{}^6\text{Li}$, ${}^{11}\text{B}/{}^{10}\text{B}$, Li/Be and B/Be production ratios higher than that of GCR (Table 2). Its main advantage is that the energy injected in the form of nuclei that is required to produce the γ -ray luminosity is $\sim 10^{39}\text{ erg s}^{-1}$, in good agreement with other calculations²¹.

In a first approach to integrating these ideas into a classical model of chemical evolution in the Galaxy we have assumed that stars with masses equal or greater than $60\text{ }M_{\odot}$ will explode within giant molecular clouds. The fact that the production rates are only weakly dependent on metallicity simplifies the calculations. The best fit of the Be/H and B/H ratio at low metallicity is obtained with an internal irradiation time of $5 \times 10^4\text{ yr}$, which minimizes the energetic demand²². The supernova rate is taken as proportional to the gas mass fraction in the galactic disk, as usual. The stellar initial mass function (the relative numbers of stars in each mass range) is parametrized as $f(m) \propto m^{-2.7}$, in agreement with a recent estimate¹⁰. Standard galactic cosmic rays are incorporated as in a previous work²³. Figure 1a and b shows the evolution of beryllium and boron, which avoids the overproduction of lithium at very low metallicity²⁴. An extra component, presumably related to low-mass AGB stars or novae, is required to synthesize the ${}^7\text{Li}$ observed in young stars.

A possible objection to this scenario would be that the combined γ -ray emission of all the Orion-like objects in the Galaxy could give rise to an excessive disk emission. We now estimate the total number of Orion-like regions in the disk. The total mass of boron in the Galaxy is $\sim 100\text{ }M_{\odot}$. The Orion boron production is estimated as $4 \times 10^{-6}\text{ }M_{\odot}$; therefore 2.5×10^7 "Orions" could have produced boron over the galactic lifetime (10^{10} yr). As the mean star-formation rate, assumed to be proportional to gas-mass fraction, is about three times higher than the present one, we estimate that there would be ~ 50 Orion-like objects in a steady state. (Note that this estimate is independent of the irradiation time.) Together, these objects emit $7.0 \times 10^{40}\text{ photons s}^{-1}$ at 4.4 MeV , which is barely detectable with present technology but within reach of the planned INTEGRAL mission.

Interesting consequences for isotopic anomalies in meteoritic inclusions and crystals arising from extinct radioisotopes like ${}^{10}\text{Be}$, ${}^{26}\text{Al}$, ${}^{41}\text{Ca}$ (A. G. W. Cameron, personal communication)

and ${}^{53}\text{Mn}$ will be analysed elsewhere. Specific signatures related to α -induced reactions would allow us to detect the supernova irradiation from solar proton irradiation in the T-Tauri phase. Note that the proton-induced reactions alone lead to a ${}^{26}\text{Al}$ production rate of $\sim 10^{37}\text{ s}^{-1}$, which is much less than the value previously quoted by Clayton²⁵. $\square$

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Effect of sample topology on the critical fields of mesoscopic superconductors

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THE superconducting state of a material can be suppressed by either increasing the temperature (T) or applying a magnetic field (H). For bulk samples, the form of the H - T phase boundary is mainly determined by the material itself; sample topology can be neglected because the surface-to-volume ratio is small¹. But for mesoscopic samples, this ratio becomes very large and nucleation of the superconducting state should depend strongly on the boundary conditions imposed by the sample shape, analogous to the role of the confining potential on the energy levels in the quantum-mechanical 'particle-in-a-box' problem². Here we describe measurements of the superconducting H - T phase boundary of a range of mesoscopic aluminium structures (lines, squares and square rings) which show clearly the effect of sample topology. The H - T phase boundaries determined experimentally are in excellent agreement with theoretical calculations.

TABLE 2 Production rates (10^{36} s^{-1})

Isotopes	$60\text{ }M_{\odot}$
${}^6\text{Li}$	4.7
${}^7\text{Li}$	24.5
${}^9\text{Be}$	0.6
${}^{10}\text{B}$	4.0
${}^{11}\text{B}$	27.6

The linearized Ginzburg–Landau equation, describing the nucleation of the superconducting state in the presence of a magnetic field $\mathbf{H} = \text{rot } \mathbf{A}$ (refs 1, 3) corresponds to

$$\frac{1}{2m} \left(-i\hbar \nabla - \frac{e^*}{c} \mathbf{A} \right)^2 \psi_s = -\alpha \psi_s \quad (1)$$

and is very similar to the Schrödinger equation for a particle with charge e :

$$\frac{1}{2m} \left(-i\hbar \nabla - \frac{e}{c} \mathbf{A} \right)^2 \psi = E \psi \quad (2)$$

where $\mathbf{A}$ represents the vector potential and m is the electron mass. The superconducting analogue of the particle wavefunction ψ is the superconducting order parameter ψ_s and its modulus is proportional to the density n of the Cooper pairs ($|\psi_s|^2 = \psi_s \psi_s^* \propto n$) with a charge $e^* = 2e$. The parameter α in equation (1) is given by

$$-\alpha = \frac{\hbar^2}{2m\xi^2(T)} = \frac{\hbar^2}{2m\xi^2(0)} \frac{T_c - T}{T_c} \quad (3)$$

and plays the same role for the order parameter ψ_s as the eigenvalue E for the wavefunction ψ . T_c is the Ginzburg–Landau critical temperature and $\xi(T)$ is the temperature-dependent superconducting coherence length which diverges at T_c . The lowest E value ($|\alpha|$ value) corresponds to the highest possible temperature for the nucleation of the superconducting order parameter. Therefore, the superconducting H – T phase boundary can be found by using the following simple procedure; (1) solve the corresponding Schrödinger equation, (2) take the lowest $E(H)$ state and calculate from the relation $|\alpha(T_c)| = E(H)$ the shift of the critical temperature $T_c(H)$.

The boundary of the superconducting sample determines the confinement geometry for the superconducting condensate. For a superconductor–vacuum interface the order parameter ψ_s obeys the following boundary conditions for the normal component of the superfluid velocity v_s (refs 1, 3):

$$(\mathbf{v}_s)_n = \frac{1}{m} \left(-i\hbar \nabla - \frac{e^*}{c} \mathbf{A} \right) \Big|_n \psi_s = 0 \quad (4)$$

It is therefore evident that by changing the actual sample topology, which enters equation (4), we can strongly modify the lowest energy level $E(H)$ and thus the superconducting H – T line. This is, however, possible only in mesoscopic superconducting systems, where the sample size $\mathcal{L}$ is smaller than both the coherence length $\xi(T)$ and the magnetic field penetration depth $\lambda(T)$. In this case the actual surface of the sample and the third (surface) critical field^{4,5} $H_{c3}(T)$ play a crucial role^{4,5} because a mesoscopic sample is, in fact, a sample with no interior in the sense that the thickness of the superconducting surface layer is $\xi(T) \geq \mathcal{L}$. The relevance of the sample topology was already hidden in the classical Little–Parks experiment⁶, which resulted in an oscillatory $T_c(H)$ line for thin hollow cylinders in an axial field. This $T_c(H)$ line is qualitatively different from the smooth $T_c(H)$ boundary of the corresponding bulk material. But a systematic comparison of the H – T phase boundaries for mesoscopic samples of different topology, made from the same material under the same conditions, has not been performed.

To check the effect of the topology of the mesoscopic samples on the H – T phase boundary, we prepared Al mesoscopic lines, squares and square rings (Fig. 1). The sample geometry was defined by combining electron–beam lithography and lift-off techniques. The resistance of the 25-nm-thick Al samples was measured using a four-probe technique with an a.c. resistance bridge. The normal-state resistivity $\rho \approx 4.5 \times 10^{-6} \Omega \text{ cm}$ of the Al structures as well as the resistance ratio $R(300 \text{ K})/R(4.2 \text{ K}) \approx 2.1$ indicate a pronounced metallic character. Detailed atomic force

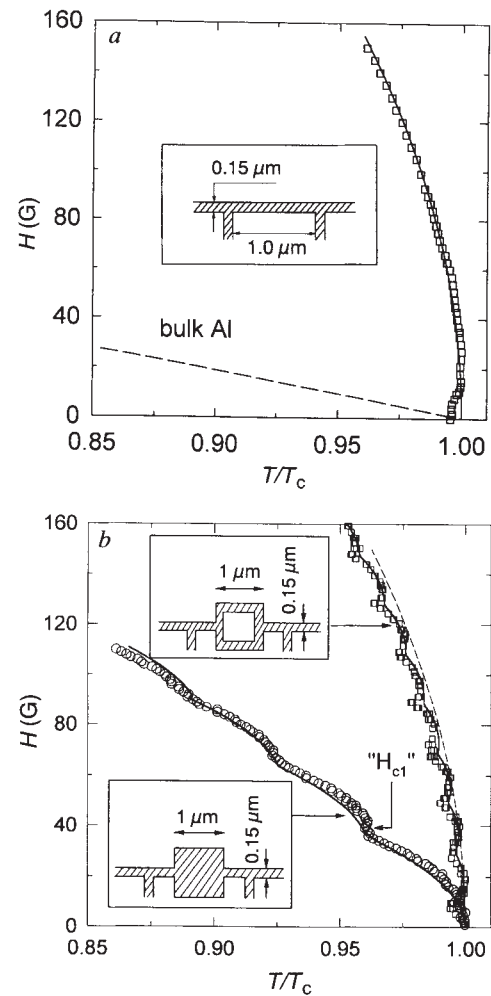


FIG. 1 a, The measured superconducting phase boundary, represented in the H – T/T_c plane, for the straight Al line with a length of 1.0 μm and a width $w = 0.15 \mu\text{m}$. The weak depression of T_c at low fields $H < 15 \text{ G}$ is due to the presence of the anomalous resistance bump, typical for mesoscopic superconducting structures^{12–14}. The solid line is calculated by using the Tinkham formula for the critical field $H_c(T)$ of a thin film (equation (5)) with $w = 0.15 \mu\text{m}$ and a coherence length $\xi(0) = 0.10 \mu\text{m}$. The dashed line represents the phase boundary for bulk Al ($H_c(T \rightarrow 0) = 100 \text{ G}$). Inset, geometry of the investigated Al line. b, The measured superconducting phase boundary in the H – T/T_c plane, for the wire interrupted by a $1 \times 1 \mu\text{m}$ Al square ring (□) and by a $1 \times 1 \mu\text{m}^2$ filled Al square (○). The solid lines correspond to calculations based on equation (6) for a ring geometry and the theoretical phase boundary found in refs 4 and 5 by solving equation (1) for a disk. For the disk the lower critical field H_{c1} corresponds to the transition between the states with orbital quantum number $L = 0$ to $L = 1$. For comparison, the dashed line shows again the phase boundary for the straight line (see a). Insets, the geometries of the square rings and filled Al squares.

microscopy and scanning electron microscopy studies of the Al films confirm the presence of a smooth and continuous film surface.

The superconducting H – T phase boundaries have been reconstructed by measuring the temperature shift of the midpoint of the normal-to-superconducting resistive transition at different magnetic fields which were always applied perpendicular to the plane of the samples. Figure 1a and b shows the H – T phase boundaries for samples with different topology. First, we discuss the experimental data for the line having a width $w = 0.15 \mu\text{m}$. The critical field shows a square-root behaviour, $H_c(T) = H_{c0}\sqrt{1 - T/T_c}$, corresponding to a shift of T_c proportional to H^2 , as demonstrated in Fig. 1a. To fit our experimental data,

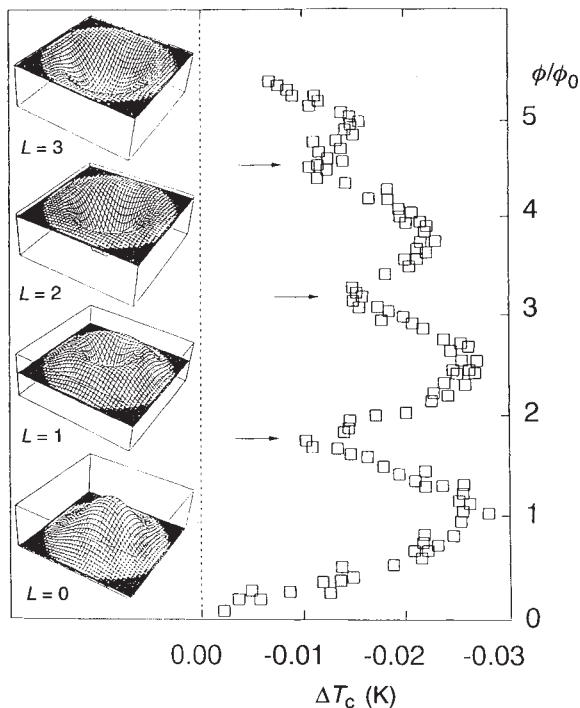


FIG. 2 The superconducting phase boundary, $\Delta T_c(H)$, for the filled Al square after subtracting the linear background (see Fig. 1b). The period of the first oscillation is noticeably larger than the period of the other oscillations (see horizontal arrows). The left-hand side of the figure shows the corresponding patterns of the order parameter $|\psi_s|^2$ for different orbital quantum number L .

we shall use the Tinkham formula⁷ for a thin slab with a thickness w in a parallel field:

$$\frac{T_c(H) - T_c(H=0)}{T_c(H=0)} = - \left(\frac{\pi \xi(0) w H}{\sqrt{3} \phi_0} \right)^2 \quad (5)$$

Here $\phi_0 = hc/2e$ is the superconducting flux quantum. It is reasonable to apply equation (5) to a narrow line in a perpendicular field as well as to a thin film in a parallel field, as the area exposed to the magnetic field has the same geometry. The theoretical $T_c(H)$ values for the line structure are in good agreement with our experimental data (see solid line in Fig. 1a), when we use $\xi(0) = 0.10 \mu\text{m}$ in equation (5). This $\xi(0)$ value has to be compared to the dirty limit value $\xi(0) = 0.85(\xi_0 l)^{1/2} \approx 0.11 \mu\text{m}$ calculated from the mean free path $l = 11 \text{ nm}$ which is obtained from the normal-state resistivity. Comparing the critical field data for the line and of the bulk Al (see the dashed line in Fig. 1a), we clearly observe a strong enhancement of the critical fields for the mesoscopic geometry.

The parabolic behaviour of $T_c(H)$ can easily be understood by using the simple concept described above, that is, solving the Schrödinger equation (equation (2)) in the weak-field limit ($\mathcal{L}H \ll 5 \text{ G cm}$; ref. 6), which is clearly applicable for a mesoscopic line (Fig. 1a). In this case the effect of the magnetic field can be obtained from perturbation theory^{8,9}, resulting in an energy level shift $\Delta E = k_1 L H + k_2 S H^2$, where L is the orbital quantum number, S is the area penetrated by the field and k_1 and k_2 are constants. The line geometry strongly favours the $L=0$ state, as other states ($L>0$) are related to a rotational motion which can not be realized for the line structure in the low field limit. Taking $L=0$ we find $[T_c(H=0) - T_c(H)]/T_c(H=0) \approx \Delta E \approx H^2$, in agreement with the classical result⁶.

Next, we consider a mesoscopic square ring (Fig. 1b). In this case we find the classical Little-Parks oscillatory $H-T$ phase boundary^{6,10}, which is related to the fluxoid quantization in the ring. To calculate the phase boundary for the ring we use the

classical equation which is obtained by averaging the one-dimensional result¹ over the different radii imposed by the finite line-width of the ring¹¹:

$$\frac{T_c(H) - T_c(H=0)}{T_c(H=0)} = - \left(\frac{\pi \xi(0) w H}{\sqrt{3} \phi_0} \right)^2 - \frac{\xi^2(0)}{R_1 R_2} \left(L - \frac{\pi H R_1 R_2}{\phi_0} \right)^2 \quad (6)$$

where R_1 and R_2 are the inner and outer radius of the ring, respectively. The first term gives a monotonic shift of $T_c(H)$ and is identical to the Tinkham formula (equation (5)) applied to a narrow line. The second term describes the oscillatory $T_c(H)$ variation caused by the change of the orbital quantum number L with increasing magnetic flux. Using the actual sample dimensions and the same $\xi(0) = 0.1 \mu\text{m}$ obtained from the fit of the data for the line (Fig. 1a), we calculate the theoretical phase boundary which agrees well with the experimental data (see solid line in Fig. 1b).

Last, Fig. 1b also shows the $H-T$ phase diagram of the filled Al square, which has the same external size as the square ring. It is clear that the suppression of the superconducting state by the field is much stronger for the filled square. This can easily be explained by the difference of the area penetrated by the magnetic field. Moreover, the period ΔH of the oscillations, superimposed on a nearly linear $H-T$ background, is larger than the period $\Delta H = \phi_0/S = 20.7 \text{ G}$ expected from the total area S of the filled square.

In order to better resolve the $T_c(H)$ oscillations of the filled square, we have subtracted the linear background and plotted the normalized magnetic flux ϕ/ϕ_0 as a function of ΔT_c in Fig. 2. The main experimental observations for the filled square (Fig. 1b and Fig. 2) can be summarized as follows: (1) the $T_c(H)$ oscillations are superimposed on a nearly linear $H-T$ phase boundary; (2) the period of the $T_c(H)$ oscillations is different from that of a square ring with the same external dimensions; (3) the period of the first oscillation, $\Delta H_1 \approx 36.4 \text{ G}$, is larger than the periods of the other oscillations ($\Delta H_2 \approx \Delta H_3 \approx \dots \approx 27 \text{ G}$). As shown in Fig. 1b, these observations are fully supported by the theoretical calculations which solve equation (1) with the proper boundary conditions (equation (4)). The field dependence of the energy levels for a disk (which we consider here as an analogue of the filled square) can be found in refs 4 and 5. These levels correspond to different orbital quantum numbers L in the solution for the order parameter ψ_s , which is the product of the radial ($f(r)$) and the angular ($e^{-il\varphi}$) dependence; $\psi_s = f(r)e^{-il\varphi}$ (Fig. 2). The solid line in Fig. 1b corresponds to the superconducting $H-T$ phase boundary, calculated from the lowest energy state, with oscillations arising from the change of the orbital quantum number L (refs 4, 5). The calculations reproduce remarkably well both the nearly linear $T_c(H)$ suppression (see the solid line in Fig. 1b) and the oscillations, including the anomalous difference between the first period $\Delta H_1 = 1.8\phi/\phi_0$ and the other periods $\Delta H_2 \approx \Delta H_3 \approx \dots \approx 1.3\phi/\phi_0$.

The mesoscopic analogue of the lower critical field H_{c1} (where the first penetration of magnetic flux occurs) for the disk can be found from the transition $L=0 \rightarrow L=1$ (Fig. 1b). Again, there is an important difference between a bulk and a mesoscopic superconductor: in the former the H_{c1} value is determined by the material properties, whereas in the latter H_{c1} is found from the fluxoid quantization condition and is strongly dependent upon the sample dimensions.

The critical field $H_c(T)$ of a line, where the geometry favours the $L=0$ state, is a smooth, non-oscillatory boundary. On the other hand, a loop, where quantized circular motion ($L \neq 0$) occurs, has an oscillatory $H_c(T)$ phase boundary. The envelope of the latter oscillatory $H_c(T)$ curve turns out to be similar to that of the simple line (see dashed line in Fig. 1b). This similarity is due to the fact that the superconducting strips forming these mesoscopic structures have the same width and are therefore subjected to the same pair breaking effect given by the first term of equation (6). Comparing the $H_c(T)$ curve for the Al square

ring, the line and the bulk Al (Fig. 1a, b), we clearly see the effects of sample topology. □

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Design and synthesis of an ultraviolet-transparent nonlinear optical crystal $\text{Sr}_2\text{Be}_2\text{B}_2\text{O}_7$

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POWERFUL, tunable ultraviolet laser sources, required for many spectroscopic applications, rely on the frequency-doubling of lasers in the visible range. Such second-harmonic generation (SHG) depends on the development of nonlinear optical (NLO) materials with high SHG coefficients, a wide transparent region in the ultraviolet, and moderate birefringence. Few such materials are available; those most widely used at present are the inorganic crystals $\beta\text{-BaB}_2\text{O}_4$ (BBO)¹ and LiB_3O_5 (LBO)². These crystals are effective for SHG only down to wavelengths of 205 nm (ref. 3), because of a limited ultraviolet-transparent range in the former case and birefringence problems in the latter. We have developed previously⁴ a new inorganic NLO crystal, $\text{KB}_2\text{BO}_3\text{F}_2$ (KBBF), which avoids both of these problems to a large degree. But it is hard to grow large crystals of KBBF because of weak binding between its layered structural units. We have now developed an improved material by rational design: $\text{Sr}_2\text{Be}_2\text{B}_2\text{O}_7$ (SBBO), which shares (and in fact slightly improves on) all of the favourable NLO properties of KBBF and is easy to grow as large (so far up to $7 \times 7 \times 3$ mm) crystals of high optical quality. The large SHG coefficients and wide range of ultraviolet transparency make this material a promising candidate for frequency doubling into the ultraviolet.

Our previous theoretical studies^{5–7} of layered borate materials such as KBBF showed that the active group responsible for the NLO properties is the BO_3^{3-} anion. The ultraviolet-transparent range of such materials is determined mainly by the energy gap of the anionic groups⁶ if the cations are alkali or alkaline-earth metals (which have no electronic transitions in this range). Thus, as the energy gap of the $(\text{BeO}_3\text{F})^{5-}$ group in KBBF is larger than that of the BO_3^{3-} ions, the latter determine the transparency in the ultraviolet. Figure 1 shows the electronic structure of the

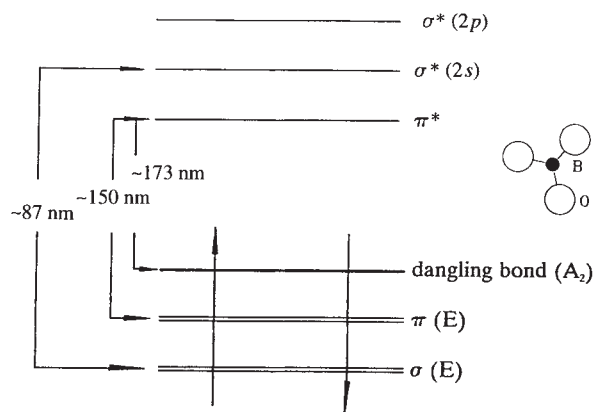


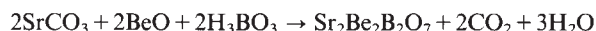
FIG. 1 Electronic structure of the $(\text{BO}_3)^{3-}$ group.

BO_3^{3-} ion calculated by the DV-SCM- X_α quantum-chemical method⁸. Absorption in this group occurs at 173 nm.

If, however, the dangling bonds of the three terminal oxygen atoms are interlinked with cations such as Be, the absorption band shifts to 150–160 nm. These considerations led us to develop KBBF (ref. 9), which contains Be_2BO_3 sheets of precisely this structure with all of the triangular BO_3^{3-} units perpendicular to the z axis of the crystal. The transparent range of this material does indeed extend down to 155 nm. The coplanar arrangement of the BO_3^{3-} groups also confers moderate birefringence (unlike LBO, for which the birefringence is small). This gives KBBF a very wide spectral range, from the visible to the deep ultraviolet (<184.7 nm), over which the phase velocities of the fundamental and second harmonic frequencies are matched¹⁰.

The layered structure of KBBF makes the crystals rather fragile and difficult to grow, however. We rationalized that this problem might be overcome by developing a similar material in which the layers are linked via strong covalent bonds, for example through bridging oxygen atoms.

Our systematic studies of the synthesis of boron–beryllium compounds have now enabled us to synthesize a compound that possesses these design elements, $\text{Sr}_2\text{Be}_2\text{B}_2\text{O}_7$. We obtained this material from the solid-state reaction:



The starting compounds, all spectrally pure, were mixed homogeneously in the appropriate molar ratios and roasted at 950°C for 48 h. The solid product was then pulverized and ground into a fine powder. This was shaped under pressure into a solid block, which was heated at 950°C for 60 h, producing the raw SBBO

TABLE 1 X-ray crystal data for $\text{Sr}_2\text{Be}_2\text{B}_2\text{O}_7$ at 296 K

Atom	Positional parameters			Isotropic thermal parameters
	x	y	z	
Sr(1)	0	0	0	0.8488 (4)
Sr(2)	0	0	1/4	0.7602 (2)
O(1)	1/3	2/3	0	0.7819 (3)
O(2)	0.6289 (3)	0.0221 (2)	0.1382 (8)	0.2063 (1)
B(1)	2/3	1/3	0.1279 (3)	–0.2527 (6)
Be(1)	1/3	2/3	0.1066 (4)	0.6605 (8)

Space group, $P6_3c$ (hexagonal system; D_{3h}^2 , no. 188 cell parameters, $a=4.683$ (3) Å, $c=15.311$ (7) Å, $z=2$; range of 2θ , 40–70; no. of observations*, 480; no. of variables, 27; unweighted agreement factor (R), 0.043; weighted agreement factor (R_w), 0.063; highest peak in final difference map, $5.6 \text{ e } \text{\AA}^{-3}$.

* Peaks with intensities $>3\sigma$.

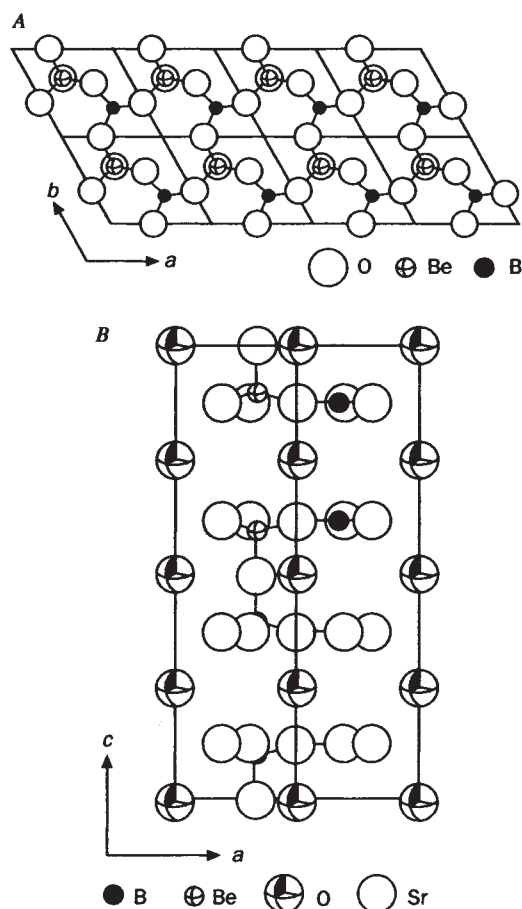


FIG. 2 Crystal structure of SBBO. A, Network structure of $(\text{Be}_3\text{B}_3\text{O}_6)_\infty$ layer. B, $(\text{Be}_3\text{B}_3\text{O}_6)_\infty$ multi-layer structure with bridging oxygen atoms bonded to beryllium atoms of adjacent layers.

material. We then used a high-temperature flux method to grow single crystals from the fused material.

We have performed X-ray crystallography on a high-optical-quality SBBO crystal $0.3 \times 0.7 \times 1.1$ mm in size, using a Rigaku AFC5R CAD-4 automated diffractometer. The structure was solved using direct methods and the TENSAN program⁷, and refined by full matrix least-squares techniques with isotropic or anisotropic thermal parameters for all atoms⁷. Table 1 lists the crystallographic data.

The basic structural features of the SBBO crystal are shown in Fig. 2. The favourable structural features of KBBF (ref. 11) are maintained in the SBBO structure, namely the nearly planar $(\text{Be}_3\text{B}_3\text{O}_6)_\infty$ network with all BO_3^{3-} groups perpendicular to the c axis, and the Be bridges between the three terminal O atoms of the BO_3^{3-} groups. The structure of SBBO also has two additional, advantageous features. First, the unit cell is more compact: the packing density of the BO_3^{3-} groups is about twice that in KBBF. This makes the SHG coefficient of SBBO about twice as large as that of KBBF (see below). Second, the binding between the $\text{Be}_3\text{B}_3\text{O}_6$ layers is stronger, as they are bridged by oxygen atoms bound to the Be. We find that this makes the crystals not only easier to grow, but also easier to cut and polish.

By using a top-seeding high temperature flux method, with starting materials similar to the above but a growth temperature of $\sim 1,100^\circ\text{C}$, we have grown SBBO single crystals $7 \times 7 \times 3$ mm in size, with very good optical quality, which can satisfy the requirements for optical and mechanical measurements. To obtain this high optical quality, close control of temperature ($\sim 0.1^\circ\text{C}$) and a very slow rate of temperature reduction ($1\text{--}2^\circ\text{C}$ per day) are needed. We find that SBBO is transparent down to ~ 155 nm (Fig. 3). The measured SHG coefficient of SBBO, d_{22} , is 1–1.5 times that of BBO and about twice that of KBBF (Table 2).

Phase-matching measurements also show the potential of SBBO for NLO applications. Efficient SHG was observed for wavelengths from 2 to $0.2\ \mu\text{m}$. The phase-matching angle of fourth-harmonic generation for a Nd:YAG laser is $\theta_{\text{pm}} \approx 40^\circ$,

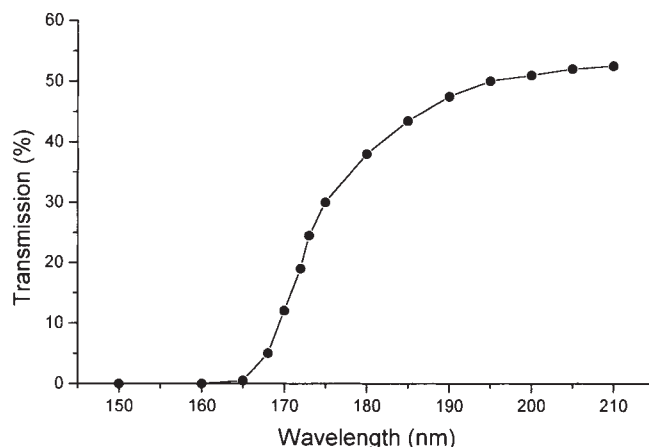


FIG. 3 Transmission spectrum of SBBO in the ultraviolet.

TABLE 2 Optical parameters of nonlinear optical materials

Crystal	Point group	Transparent range (μm)	d -coeff.* (pm V^{-1})	$d_{\text{eff}}^\dagger$	Shortest SHG wavelength (nm)
SBBO	D_{3h}	$\sim 0.155\text{--}3.78$	$d_{22} \approx 2.0\text{--}2.48$	$-d_{22} \cos \theta \sin 3\phi$ (type I)	< 200
KBBF	D_3	$\sim 0.155\text{--}3.66$	$d_{11} \approx 0.78\text{--}0.98$	$d_{11} \cos \theta \cos 3\phi$ (type 1)	< 184.7
BBO	C_{3v}	$0.190\text{--}2.60$	$d_{22} = 2.2$ $d_{31} = 0.07d_{22}$	$d_{31} \sin \theta$ $d_{22} \cos \theta \sin 3\theta$ (type I) $d_{22} \cos^2 \theta \cos 3\theta$ (type II)	205
LBO	C_{2v}	$0.155\text{--}2.6$	$d_{31} = 0.85$ $d_{32} = -0.67$	$\sim d_{31} \cos \phi$ (type I, $\theta = 90^\circ$)	≤ 277
KDP	D_{2d}	$0.20\text{--}2.0$	$d_{36} = 0.37$	$d_{36} \sin \theta \sin 2\phi$ (type I) $d_{36} \sin 2\theta \cos 2\phi$ (type II)	266

Abbreviations: SBBO, $\text{Sr}_2\text{Be}_2\text{B}_2\text{O}_7$; KBBF, $\text{KBe}_2\text{B}_3\text{O}_6\text{F}_2$; BBO, $\beta\text{-BaB}_2\text{O}_4$; LBO, LiB_3O_5 ; KDP, KH_2PO_4 .

* The d_{ij} notation is a two-dimensional representation of the coefficients of the three-dimensional tensor that characterizes the second-order nonlinear optical response of an anisotropic crystal. The coefficients cited for each material are those for which the condition of phase matching is satisfied (a prerequisite for efficient SHG), which in turn depends on the symmetry group of the crystal.

† Effective second harmonic generation (SHG) coefficients. θ is the angle between the z axis of the crystal and wave vector $\mathbf{k}$ of the incident laser beam, ϕ is the angle between the projection of $\mathbf{k}$ onto the xy plane and the x axis of the crystal (the azimuthal angle). The conditions for phase matching depend on the polarization states of the two interacting fundamental light waves—they may be parallel (type I) or orthogonal (type II).

which is 7° smaller than that of BBO. So the effective SHG coefficient, d_{eff} , of SBBO (Table 2) in the ultraviolet is always larger than those of BBO and LBO. It is clear from Table 2 that SBBO compares very favourably with other standard materials (BBO, LBO, KBBF and KDP) (KH_2PO_4) used for NLO applications. $\square$

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A 'smart' catalyst that self-assembles under turnover conditions

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VIRTUALLY all synthetic materials with a dynamic function, from catalysts to integrated circuit elements, degrade irreversibly with time. The inevitability of decay is an implicit consideration in the design of materials or molecules that serve these functions, and fabrication methods tend to aim simply at minimizing the rate of decay. Here, by contrast, we describe a molecular catalyst that experiences a thermodynamic and kinetic driving force for its own reassembly and repair under the conditions of catalysis. We show that the multicomponent polyanion cluster $\alpha\text{-}[(\text{Co}^{\text{II}})\text{PW}_{11}\text{O}_{39}]^{5-}$ self-assembles from four precursor species, containing a total of 28 molecules, and that as it assembles it starts simultaneously to catalyse the epoxidation of alkenes with high selectivity. This conclusion follows from the observation that the kinetics of self-assembly and those of catalysis are closely correlated as the reactions proceed. Should it be fragmented during operation, this polyanion catalyst will therefore experience a thermodynamic and kinetic driving force for its own repair.

The basic concept depends on fundamental kinetic principles and is illustrated in reactions (1) and (2) (Fig. 1). Conditions are found under which two important events, catalyst self-assembly (reaction (1)) and catalysis by the assembling catalyst (reaction (2)), take place simultaneously. For this experiment to be effective, the following points are required: first, all key components in both reactions (1) and (2) must be quantified spectroscopically on timescales faster than these reactions to facilitate kinetic analysis. It is helpful that none of the components individually catalyse reaction (2) on this timescale (reaction (3) in Fig. 1). Second, conditions for reactions (1) and (2) must be very similar, that is, nothing present only in reaction (1) can markedly perturb reaction (2) and *vice versa*. It assists kinetic resolution if reaction (2) is substantially faster than reaction (1). One significant result of the simultaneous occurrence of reactions (1) and (2) is that if the catalyst is damaged and fragmented into its synthetic precursors (the reverse of reaction (1)) during catalysis, there exist forces for its regeneration, and thus the catalyst exhibits a primitive 'intelligence' regarding its integrity. This work is conceptually

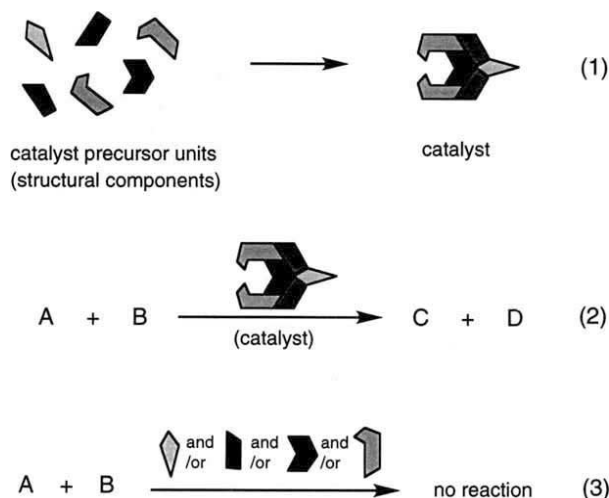
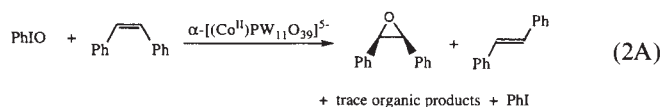
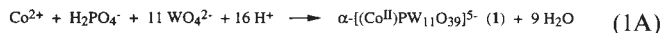


FIG. 1 Basic concept behind the 'smart' catalytic system, showing reactions (1), (2) and (3).

ally and motivationally distinct from the elegant self-replication studies of Rebek and co-workers^{1,2}. It also builds on studies to optimize the stability of catalyst components, including thorough optimization of soluble V–Mo polyoxoanions with Pd co-catalysts, or catalysts themselves (heterogeneous oxidation catalysts)^{3,4}. Here we demonstrate that a total homogeneous catalytic system can be kinetically and intrinsically stable.

The realization of reactions (1) and (2) is given in reactions (1A) and (2A), respectively. Reaction (1A) involves self assembly of the Co-substituted polytungstophosphate^{5,8}, $\alpha\text{-}[(\text{Co}^{\text{II}})\text{PW}_{11}\text{O}_{39}]^{5-}$, **1** (Fig. 2), which is a selective catalyst for the epoxidation of alkenes under the same conditions, reaction (2A):



Oxidations are some of the most intensely investigated and commercially significant homogeneous catalytic reactions^{9–21}. Complex **1** and reaction (2A) were specifically chosen for this study as **1** is totally inorganic and consequently oxidatively resistant^{22–29} and reaction (2A) is relatively selective and very long lived (no detectable damage to **1** after 10,000 turnovers). After an extensive and systematic evaluation of the reaction variables involved in both reactions (1A) and (2A), appropriate conditions, given in Fig. 3 and Table 1, were found to satisfy all the criteria given above. The actual experimental procedure involved running reaction (1A) in one flask for a total of 25 min and during the process of forming **1**, aliquots of this solution were transferred to a series of identically prepared flasks, each containing the reactants for reaction (2A) and the phase transfer agent, cetylpyridinium chloride, at set elapsed reaction times of 1, 6, 11 and 16 min. Curves for the products of reaction (2A) versus time were generated for each aliquot of forming catalyst for a constant catalytic reaction evaluation time of 4 min. Each product–time curve yielded both rates and catalytic selectivity data for the epoxidation. Figure 3 thus compares the kinetics for reaction (1) (**1** versus time), with initial rates and selectivities versus time. Additional experimental details are given in Fig. 3 legend. Measurements indicated no change in the structural or catalytic properties of **1** after several weeks.

Figure 3 clearly indicates that the time-dependence of formation of **1** (reaction (1A)) and its catalytic profile for oxidation

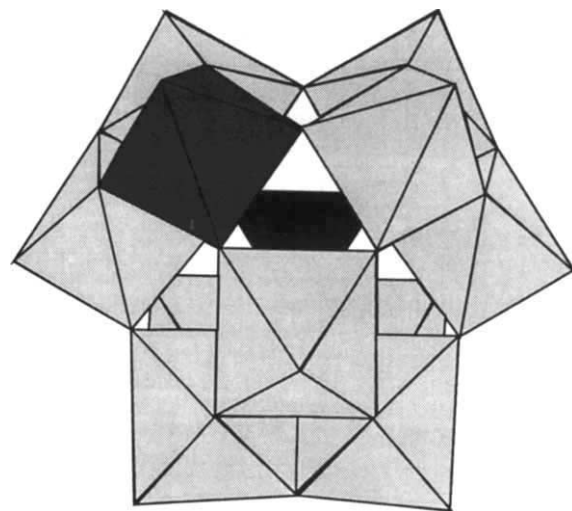
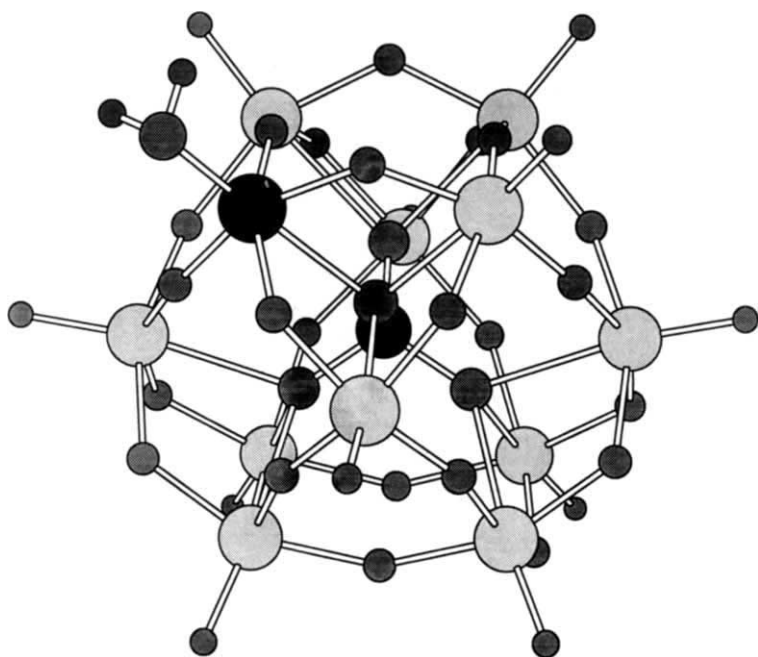


FIG. 2 The C_s symmetry, 1.1-nm diameter, polyoxoanion complex, α - $[(Co^{II})PW_{11}O_{39}]^{5-}$, **1** in bond (left) and polyhedral (right) notation. The $Co(II)$ ion, which is in the upper left-hand corner in both representations, is depicted as a darker sphere with a terminally coordinated H_2O

molecule in the left picture and a darker coordination polyhedron in the right picture. The $P(v)$ heteroatom resides in a central pseudo-tetrahedral hole of the structure; it is the dark central atom in the left picture and the very dark tetrahedron in the right picture.

of *cis*-stilbene correlate closely. The catalytic profile consists not only of the rate of formation of epoxide product, *cis*-stilbene oxide, and in this case, formation of isomerized alkene, *trans*-stilbene (Fig. 3a), but also the organic product selectivity (Fig. 3b). The product selectivity is defined as the concentration of the desirable epoxide product, which increases with time and increasing [1] (Fig. 3), divided by the total concentrations of all organic products. At time 0 and at minimal concentrations of [1], the rates of epoxidation and the epoxidation selectivities are

both low, and all three of these experimental parameters increase on the same timescale. The same correlation of [1] with epoxidation rates and selectivities is seen for oxidation of cyclohexene where epoxidation by pure **1** proceeds in ~100% selectivity (Table 1).

Three control experiments were conducted that provide independent confirmation that reaction (3) (Fig. 1) does not proceed. First, all the components of reactions (1A) and (2A) were mixed together and an excess of nitric acid was added. No

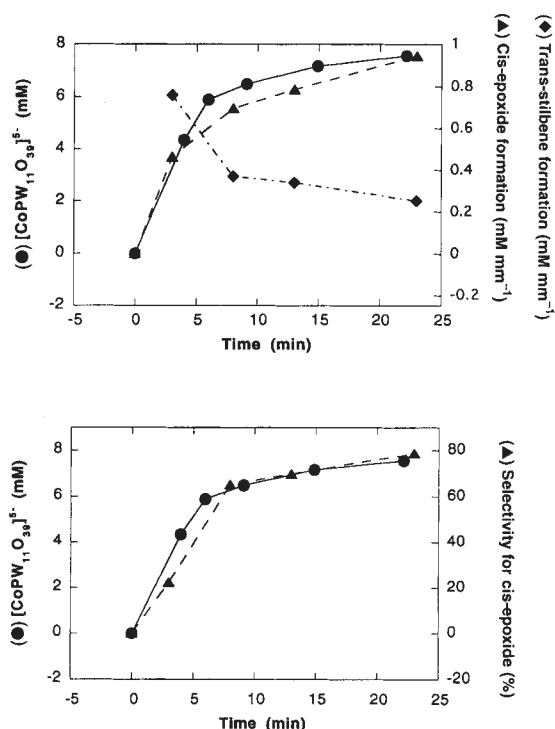


FIG. 3 Simultaneous monitoring of the catalyst, α - $[(Co^{II})PW_{11}O_{39}]^{5-}$, **1**, (by ^{31}P NMR) and its catalysis of *cis*-stilbene oxidation by iodosylbenzene (by capillary gas chromatography), as a function of time. a, Formation of **1** and initial rates of formation of the two products, *cis*-stilbene oxide and *trans*-stilbene. b, Formation of **1** and epoxide selectivity. METHODS. Conditions for formation of **1** (reaction (1A)): 5 ml of 0.37 M aqueous sodium tungstate was added to 6 ml of an acetate-buffered solution (pH = 4.8) that was 0.057 M in sodium dihydrogen phosphate, 0.028 M in cobalt sulphate and 0.38 M in nitric acid. Conditions for catalysis by **1** (reaction (2A)): 0.1 M alkene substrate in 5 ml chloroform, 0.07 M cetylpyridinium chloride (phase transfer reagent) and 0.14 g iodosylbenzene (oxidant) were placed in each of several Schlenk flasks fitted with magnetic stirring bars and rubber septa. Each reaction was degassed, placed under argon and then initiated by the addition of 1 ml of the first solution (with the catalyst, **1**, in the process of forming) at 1, 6, 11 and 16 minutes after mixing respectively. The reactions were stirred at 1,100 r.p.m. and quenched after 4 min by addition of 1 ml of saturated aqueous sodium bisulphite. Experimental controls indicate that the rate of reaction (1A) was unaffected by the presence or absence of a contacting chloroform phase containing all the reactants of reaction (2A) except iodosylbenzene, and that phase transfer of **1** to chloroform by cetyl pyridinium cation was quantitative and faster than reactions (1A) or (2A).

TABLE 1 Organic product distributions and selectivities as a function of time

Substrate	Time*	Products			
		Rate of formation, mM min ⁻¹ (selectivity, %) [†]			
					
	0	ND	ND	ND	
	1	0.689 (91.2)	0.0669 (8.8)	ND	
	6	1.48 (96.0)	0.0625 (4.0)	ND	
	11	1.68 (100)	ND	ND	
	16	1.84 (100)	ND	ND	
					
	0	ND	ND	ND	
	1	0.275 (100)	ND	ND	
	6	0.732 (100)	ND	ND	
	11	1.07 (100)	ND	ND	
	16	1.51 (100)	ND	ND	
					
	0	ND	0.223 (100)	ND	
	1	0.463 (22.4)	0.758 (36.7)	0.843 (40.8)	
	6	0.695 (65.2)	0.371 (35.8)	ND	
	11	0.786 (69.8)	0.339 (31.2)	ND	
	21	0.945 (78.9)	0.253 (21.1)	ND	

Reaction conditions: 25 °C, 1 ml of solution containing forming α -[(Co^{II})PW₁₁O₃₉]⁵⁻, 0.1 M alkene substrate in 5 ml chloroform containing 0.07 M cetylpyridinium chloride as phase transfer reagent and 0.14 g iodosylbenzene as oxidant; reactions are stirred at 1,100 r.p.m. and quenched after 4 min by addition of 1 ml of saturated aqueous sodium bisulphite.

* Elapsed reaction time and time $t=0$ are defined in the text.

[†] The organic products were quantified by gas chromatography (5% phenyl methyl silicone capillary column, N₂ carrier gas, flame ionization detection) and gas chromatography/mass spectrometry. Rate of formation given by product (mM)/reaction time of 4 min (selectivity defined as mol of particular organic product/mol of total organic products derived from alkene). ND, not detectable; below the detectable limit (<0.2%).

catalyst, **1**, forms and the overall rate is at least twenty times slower than when **1** is fully formed; the only product seen is *trans*-stilbene (that is, the selectivity for epoxide is ~0%). Second, the complete system for reactions (1A) and (2A), minus only Co²⁺ (so the catalyst, **1**, cannot form) shows some catalytic oxidation but with an approximately 20-fold slower rate and with far lower epoxidation selectivities (30 ± 3%) at all elapsed reaction times than for the complete system. Third, the complete system for reactions (1A) and (2A), minus only the tungstate precursor, WO₄²⁻, (so that **1** cannot form) shows no detectable catalytic oxidation activity (<1% relative to the full system would have been detectable). This final control experiment is used as the time $t=0$ point in Table 1 and Fig. 3.

The data provide evidence that reactions (1A) and (2A) constitute an example of a catalyst with dynamic and not just static stability. Such stability should remain provided that the collective reaction conditions of the system do not change significantly. Although the impetus for this research, in part, was and is to develop intrinsically better and more resilient catalysts, these ideas should assist the formulation of other more resilient materials with dynamic functions. □

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Changes in oceanic and terrestrial carbon uptake since 1982

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CHANGES in the carbon isotope ratio ($\delta^{13}\text{C}$) of atmospheric CO₂ can be used in global carbon-cycle models^{1–5} to elucidate the relative roles of oceanic and terrestrial uptake of fossil-fuel CO₂. Here we present measurements of $\delta^{13}\text{C}$ made at several stations in the Northern and Southern hemispheres over the past decade. Focusing on the highest-quality data from Cape Grim (41° S), which also provide the longest continuous record, we observe a gradual decrease in $\delta^{13}\text{C}$ from 1982 to 1993, but with a pronounced flattening from 1988 to 1990. There is an inverse relationship between CO₂ growth rate⁶ and El Niño/Southern Oscillation (ENSO) events which is not reflected in the isotope record. Thus, for the ENSO events in 1982, 1986 and 1991–92, we deduce that net ocean uptake of CO₂ increased, whereas during La Niña events, when equatorial sea surface temperatures are lower, upwelling of carbon-rich water increases the release of CO₂ from the oceans. The flattening of the trend from 1988 to 1990 appears to involve the terrestrial carbon cycle, but we cannot yet ascribe firm causes. We find that the large and continuing decrease in CO₂ growth starting in 1988⁶ involves increases in both terrestrial and oceanic uptake, the latter persisting through 1992.

We employ a simplified version of the global carbon and ¹³C budgets developed by Tans *et al.*⁴, to explore changes in $d(\delta^{13}\text{C})/dt$, where t is time. The budget relies on the fact⁷ that the product

of total carbon and its isotopic ratio ($C \times \delta^{13}C$) is a conservative quantity in the atmosphere. If the rate of change of total atmospheric carbon (C_a) is expressed as:

$$\frac{d}{dt}(C_a) = F_f + N_s + N_b \quad (1)$$

where F_f is the fossil-fuel release, N_s is the net ocean exchange and N_b the net plant exchange, then a similar presentation of a $C \times \delta^{13}C$ budget is possible:

$$\begin{aligned} \frac{d}{dt}(C_a \delta_a) &= \delta_a \frac{d}{dt}(C_a) + C_a \frac{d}{dt}(\delta_a) \\ &\approx F_f \delta_f + N_s(\delta_a + \epsilon_{as}) + N_b(\delta_a + \epsilon_{ab}) \\ &\quad + G_s(\delta_a^s - \delta_a) + G_b(\delta_a^b - \delta_a) \end{aligned} \quad (2)$$

where the four last terms represent, respectively, the fossil-fuel release, net ocean exchange, net plant exchange and the isotope disequilibria. Here subscripts f, a, s and b refer to fossil, atmosphere, sea and land biota respectively. We express the atmospheric carbon content C_a in units of Gt C (10^{15} g of carbon), and isotopic ratio, δ_a , in ‰ relative to V-PDB- CO_2 . The annual net fluxes, F_f , N_b , N_s are in Gt C yr⁻¹ and ϵ_{as} , ϵ_{ab} represent the isotopic shifts that occur on air-sea and air-plant exchange, respectively. The convention adopted is that the fossil-fuel emission, N_f , is a positive number, so that ocean and terrestrial uptake are $-N_s$ and $-N_b$ respectively. The gross flux (G), or disequilibrium, terms become important when the annual average isotopic values of air-to-reservoir fluxes (δ_a) are different from that of the reservoir-to-air fluxes (δ_a^s). Global disequilibria result from two conditions: the changing atmospheric value (decreasing $\delta^{13}C$ due to fossil-fuel and terrestrial releases), and the finite residence time in the terrestrial and oceanic reservoirs. Disequilibria might also occur if the kinetic fractionation factors between the atmosphere and the surface reservoirs (ϵ_{as} , ϵ_{ab}) change.

We use known fossil-fuel releases and measured $d(C_a)/dt$ in equation (1) to obtain $(N_s + N_b)$. A second relationship between N_s and N_b comes from equation (2), after assumptions about the disequilibrium terms. The implicit assumption in past work has been that the disequilibrium terms are relatively constant; then the difference between the terrestrial photosynthetic and air-sea diffusion fractionation with $\epsilon_{ab} \approx 10\epsilon_{as}$ means that changes in N_b are effectively determined by changes in $d(\delta^{13}C)/dt$. This work does not extend information on uncertainties in mean disequilibrium values beyond that already presented²⁻⁴. We simply normalize to estimates appropriate to 1986–87 adapted from Enting *et al.*^{2,3} and summarized in Fig. 2 legend.

Figure 1 shows the $\delta^{13}C$ data used here. The Cape Grim record, which alone uses *in situ* extraction⁸ of CO_2 , exhibits a distinct flattening in the global $\delta^{13}C$ trend occurring from early 1988 to late 1990. The global extent of this anomaly is evident at the other stations, all employing whole-air flask sampling, and including recent data from the NOAA/CU network⁶. Around 1991–92, the Southern Hemisphere stations suggest a partial resumption of the long-term decrease, not so evident in the north.

Figure 2a shows the $\delta^{13}C$ trend for Cape Grim over 12 years (including 1993), and for global composites from 1984 to 1992. For the Cape Grim record, with high precision and data density and small seasonality, we employ two-year smoothing⁹; for other stations we use three-year smoothing. The smoothing accommodates adjustment processes between the two hemispheres (in the sense that regional trend variations are unlikely to be maintained for more than 1–2 years in the presence of global atmospheric mixing), the relative sparseness of stations and the sparseness of data at some stations. Because of the desire for the longest possible record, and because of the sensitivity of a composite slope to normalization procedures, we present the Cape Grim data as a candidate for the best available record of the global behaviour

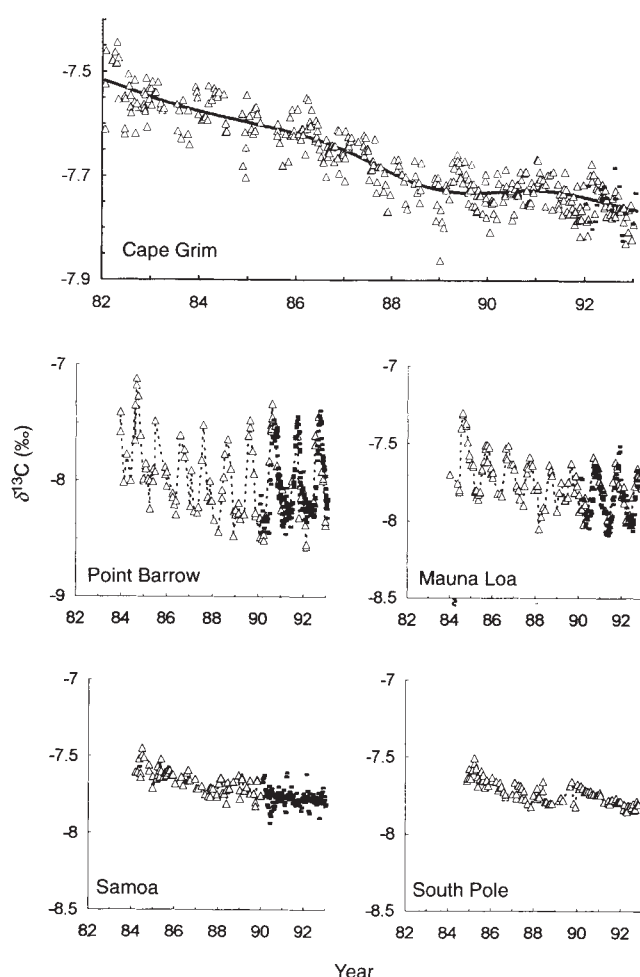
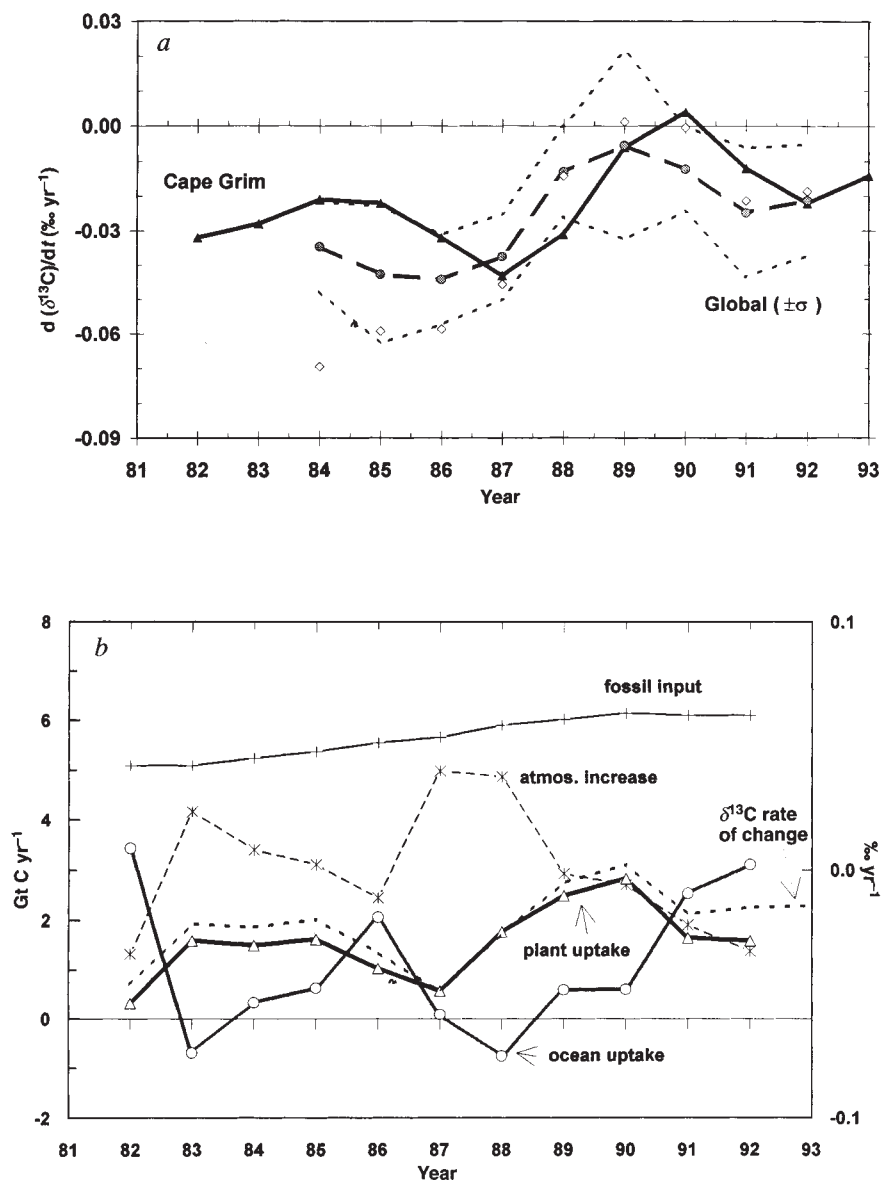


FIG. 1 Measurements of $\delta^{13}C$ in CO_2 from the CSIRO (triangles) and NOAA/UC (bars) programmes at Point Barrow (71° N), Mauna Loa (21° N), Samoa (14° S), Cape Grim (41° S) and the South Pole (90° S). The NOAA/UC samples are analysed at the University of Colorado (M.T. *et al.*, manuscript in preparation). All data come from whole-air flask collections (CSIRO samples dried) except the long-term Cape Grim record, which is based on two-hour *in situ* CO_2 extraction, approximately weekly, from marine air in conditions of strong westerly winds (44 ± 15 km h⁻¹, from $240 \pm 25^\circ$ N). All CSIRO measurements have been made relative to pure CO_2 with assigned carbon isotopic value, $\delta^{13}C = -6.396\text{‰}$, close to the air value¹¹. The curve through the Cape Grim data is digitally filtered deseasonalized data with 50% FWHM (full-width at half-maximum) attenuation at two years, to emphasize the flattening of the long-term trend. Time marks represent 1 January of the years 1982–92. (The CSIRO data are available from R.J.F.)

of $\delta^{13}C$ over the full decade. This strategy preserves special advantages, in particular the unusual degree of continuity in the Cape Grim methodology and calibration procedures, two-hour sample integration and relatively high sampling rate^{10,11}. The continuous use of one working reference gas with a $\delta^{13}C$ very close to the air value emerges as the single most effective measure in minimizing potential calibration drifts in the CSIRO programme (C. Flehocz, R.J.F., M. Leuenberger and C.E.A., manuscript in preparation). The shortcomings associated with this strategy, in particular the question of representativeness of Cape Grim, are addressed below.

Figure 2b shows the implied changes in ocean and terrestrial uptake. Within the overall constraint of equation (1), the trend in $\delta^{13}C$ (right-hand scale) effectively indicates the changes in terrestrial uptake. Following Enting *et al.*^{2,3}, a global disequilibrium with the terrestrial reservoir increases from -0.28 to -0.36‰ over the decade, calculated using a $\delta^{13}C$ record from

FIG. 2 a, Annual average values of the trend in deseasonalized data, $d(\delta^{13}\text{C})/dt$, derived from two-year smoothing with a digital filter for Cape Grim⁹ and smoothing splines with 50% attenuation at three years for global data. Note that time marks correspond to annual average values for the year (unlike Fig. 1). Cape Grim values (filled triangles) are compared to global average values (filled circles, stations of Fig. 1). The latter, accompanied by ± 1 standard deviation curves (dashed lines), are a global average which omits Point Barrow data; diamonds show mean values including Point Barrow. Larger uncertainties result from the sparser data and larger seasonality of northern stations, but the main justification for omitting Point Barrow is on the basis of 1984–85 data quality⁸. With all data combinations, the mean annual $\delta^{13}\text{C}$ trend is significantly less than zero before 1987 and close to zero for the years 1988–90. b, Changes in global average ocean ($-N_s$) and terrestrial ($-N_b$) uptake determined from the Cape Grim $d(\delta^{13}\text{C})/dt$ using equations (1) and (2). Fossil fuel emissions ($\pm 0.2 \text{ Gt C yr}^{-1}$, 1992 value assumed⁶, atmospheric carbon accumulation rate ($\pm 0.4 \text{ Gt C yr}^{-1}$)⁶ and derived net air–sea and air–plant carbon fluxes relate to the left-hand axis. The $\delta^{13}\text{C}$ rate of change ($\pm 0.005\text{‰ yr}^{-1} \cong \pm 0.3 \text{ Gt C yr}^{-1}$)^{2,3} relates to the right-hand axis, and largely determines the change in net terrestrial exchange. The combined errors lead to typical uncertainties of $\pm 0.7 \text{ Gt C yr}^{-1}$ in the annual flux estimates. In addition, the mean level of each uptake flux has an extra $\pm 1 \text{ Gt C yr}^{-1}$ uncertainty^{2,3} due mainly to disequilibria. The parameters in equations (1) and (2) appropriate to 1987 used here were as follows: δ_f , δ_a , ϵ_{as} , $\epsilon_{ab} = -28.2$, -7.71 , -1.8 , -18‰ ; $F_f = 5.70 \text{ Gt C yr}^{-1}$, $C_a = 739 \text{ Gt C}$; $G_s(\delta_a^s - \delta_a)$, $G_b(\delta_a^b - \delta_a) = -43.8$, $-25.8\text{‰ Gt C yr}^{-1}$. The revised value of δ_f reflects a recent reassessment (R.J. Andres, G. Marland, T. Boden and S. Bischoff, personal communication). The most uncertain parameter, the global isotopic disequilibrium with the oceans ($G_s(\delta_a^s - \delta_a)$), is derived from the calculations of Tans *et al.*⁴, adjusted in time (thus the mean fluxes reflect the 'low ocean uptake' range of contemporary budget estimates); note however that mean ocean uptake is increased over the estimates of Tans *et al.* owing to an increased biotic disequilibrium term^{2,3}.



air and ice cores¹² and a box model of the terrestrial biota¹³. Using the same ice-core record of $\delta^{13}\text{C}$, a box model of ocean carbon predicts a 0.15‰ increase in the air–sea disequilibrium over the decade (P. Monfray and O. Aumont, personal communication). Without the slowly increasing disequilibrium corrections, the net terrestrial uptake in 1992 relative to 1982 would be $\sim 1.2 \text{ Gt C yr}^{-1}$ less.

The high terrestrial uptake from 1988 to 1990–91 (corresponding to the $\delta^{13}\text{C}$ flattening) is a feature of Fig. 2b. Apart from 1987 and 1988, there is a tendency for a weak positive correlation between the atmospheric growth rate and the terrestrial uptake ($-N_b$), the opposite to that expected if the terrestrial reservoirs were controlling the CO_2 level. The budgeting therefore relegates this role to the oceans, so that Fig. 2b shows an overall marked anticorrelation between ocean uptake and atmospheric growth rate.

The global trends in $\delta^{13}\text{C}$ of Fig. 2a differ from the 1978–89 record compiled by Keeling *et al.*¹ by several times the quoted uncertainties of the respective programmes, and the difference is too large to have a biogeochemical explanation. The data sets

are compared in Fig. 3, in terms of differences from the 1982–92 means, using the extended normalized 'carbon flux anomalies' of Whorf *et al.*¹⁴, and normalized data from Fig. 2b. We have also included results using our global $\delta^{13}\text{C}$ trend of Fig. 2a. Before 1990, isotopic variation (not seen in our data) forces large terrestrial flux anomalies in the Whorf *et al.*¹⁴ curve. Their large out-of-phase ocean fluxes reflect the budgeting requirement to offset the terrestrial fluxes. Comparable data collected from 1984 using aircraft over Japan¹⁵ exhibit similar features to the CSIRO surface data, including the obvious flattening from 1988, but with an initial small positive $\delta^{13}\text{C}$ slope (enhanced terrestrial uptake) between 1984 and 1985.

The typical statistical uncertainties in the three input curves to the Fig. 2b budget, discussed in the Fig. 2b legend, are relatively small. However there are ill-defined, potentially large, biases that could alter the conclusions. Two are discussed here, the representativeness of the Cape Grim $\delta^{13}\text{C}$ record, and the possibility of interannual variation in the gross flux (disequilibrium) terms.

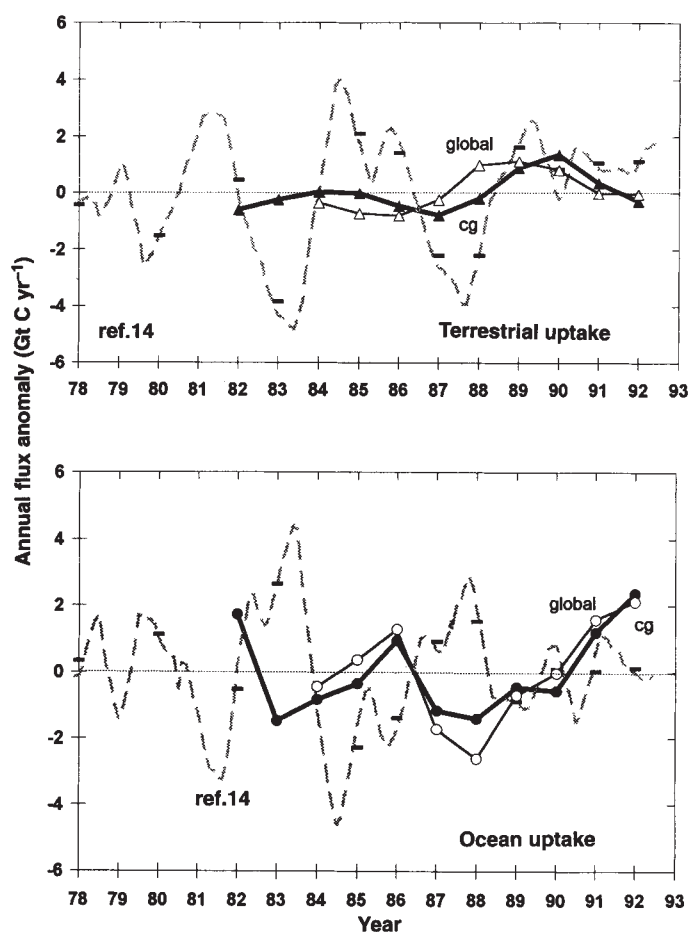


FIG. 3 Terrestrial (top panel) and ocean (bottom panel) uptake annual flux anomalies (differences from a 1982–92 average) derived for both Cape Grim only (heavy solid line labelled 'cg'), and CSIRO global data (solid line; 'global'). The uncertainty on an annual value is typically ± 0.5 Gt C yr $^{-1}$. These are compared with 'flux anomalies' (dashed lines) derived from smoothed South Pole (90° S) – Mauna Loa (21° N) average $\delta^{13}\text{C}$ of Whorf *et al.*¹⁴. The data of ref 14, which extend the data of ref. 1, are deviations from a predicted anthropogenic release scenario and are also normalized so that the 1982–92 flux is zero; annual averages are also indicated (horizontal bars). Time labels correspond to annual average or 'mid-year' values. (Note that application of our methodology to the Keeling *et al.*¹ $\delta^{13}\text{C}$, only available until 1988, gives essentially the same result, but with a small 'rotation' resulting from our inclusion of changing isotope disequilibrium).

Even if we accept that the calibration of the Cape Grim record is correct, the representatives of Cape Grim remains problematic within limits defined by the Cape Grim–global differences (of Fig. 2a). Cape Grim generally exhibits smaller trends than the global average, except in 1987–88. There is a tendency for the high Northern Hemisphere sites (for example, Barrow Point) to exhibit the largest trend variation, and additional NOAA data suggest that this is a widespread Northern Hemisphere phenomenon (J.W.C.W. and M.T., unpublished data). It is still difficult to know to what degree this reflects regional rather than zonal behaviour. By the same token, interannual variation in CO₂ vertical gradient at Cape Grim has been reported¹⁶ and possible influences on isotopic profiles, though small, are still to be thoroughly examined. In Fig. 2a, annual differences are of the order of $\pm 0.015\text{‰ yr}^{-1}$ (± 1 Gt C yr $^{-1}$) which we mostly attribute to uncertainties in the northern flask data (large seasonality, sparse data). In all of the data sets the 1988 to 1990–91 flattening anomaly is prominent. But we will first address possible explanations for the overall decrease in $\delta^{13}\text{C}$ over the decade, using the Cape Grim record and addressing the possible biases that this introduces.

The reduced $\delta^{13}\text{C}$ trend is in the sense of increased disequilibrium terms. We used three-year average flux values for the 1985–87 and 1988–90 periods to investigate sensitivity to interannual variation in the disequilibrium terms. The changes required to maintain the 1988–90 N_b at the 1985–87 level of -1.14 Gt C yr $^{-1}$ are 45%, 77% respectively in $G_s \times (\delta_a^s - \delta_a)$ or $G_b \times (\delta_b - \delta_a)$. These changes appear to be unrealistically large for any one gross flux or disequilibrium change. For example, a 45% change in $(\delta_a^s - \delta_a)$ is considered the most likely possibility and this could change via the equilibrium fractionation factor. The temperature coefficient¹ of $-0.115\text{‰ per degree C}$ requires a global three-year average sea surface temperature change of -2°C , whereas global sea surface temperature anomalies¹⁷ were $\sim +0.1^\circ\text{C}$ during the 1983 ENSO, and $+0.2^\circ\text{C}$ in late 1987, with duration typically one year. Further quantitative assessment of these factors is outside the scope of this work, other than to comment that the required change in any one of the disequilibrium parameters appears to be unrealistically large as a global 1–3 year average.

We therefore focus on an explanation for the $\delta^{13}\text{C}$ trends in terms of net fluxes. A most encouraging aspect of Fig. 2b is the relatively independent nature of the implied ocean and terrestrial fluxes, as a 1:1 anticorrelation is the inevitable consequence of errors in isotopic trend estimates. (The 1991–92 changes are the most suspicious in this regard, possibly as a result of end effects, and should be treated with extra caution.) The large ocean uptakes for 1982, 1986 and 1991–92 correspond to the early phase of warm ENSO events¹⁸. It may well be that the presence of warm water in the equatorial Pacific at these times implies reduced carbon release from the upwelling of cold carbon-rich deep water, implying (other fluxes being unchanged) an increased net global uptake. The low ocean uptake/high CO₂ growth corresponds to cool periods of equatorial sea surface temperature (La Niña), with upwelling and release of CO₂ in equatorial regions. The terrestrial uptake exhibits small decreases in ENSO years or shortly after, which if real, are consistent with climate-induced reductions in terrestrial photosynthesis or increases in biomass burning. Two full ENSO cycles are apparent in $-N_s$ and $-N_b$, suggesting that equatorial ocean fluxes dominate the global CO₂ levels. Note, however, that for 1986, in which the ENSO change is best determined, the global CO₂ ocean flux changes from ~ 0.5 to 2 Gt C yr $^{-1}$ favouring the larger estimates of CO₂ fluxes associated with ENSO events¹⁹. Although the mean level of the flux estimates from the atmospheric record are uncertain by around ± 1 Gt C yr $^{-1}$ due mainly to the disequilibrium^{2,3}, the change is determined to ~ 0.4 Gt C yr $^{-1}$. Over the last decade in the equatorial Pacific, net annual reduction of the ocean–atmosphere CO₂ flux during El Niño was of the order of 0.4 – 0.8 Gt C yr $^{-1}$ (ref. 20). Thus our results suggest that range of evasion rates is underestimated or that there is a contribution to the global oceanic flux changes outside the equatorial Pacific.

The main contrast between the two ENSO cycles lies with the $\delta^{13}\text{C}$ flattening from 1988 to 1990. Following the discussion above, we interpret this—the most robust feature of the combined isotope data—in terms of increased global terrestrial photosynthetic uptake or reduced biomass burning. We initially pursued an explanation for the $\delta^{13}\text{C}$ flattening in terms of a simple causal relationship with ENSO, involving increased tropical biomass burning in 1987–88 (contributing to the high CO₂ growth, accompanying suggested tropical release anomalies⁶, and resulting in dispersal of nutrients that fertilize tropical forests in the subsequent 2–4 years). Although this scenario cannot be excluded, we have been unable to document supporting ecological evidence, and it is difficult to reconcile with CO₂ growth-rate behaviour as a function of latitude⁶.

The timing of the $\delta^{13}\text{C}$ flattening is very similar to the timing of changes in CO₂ latitudinal gradient over the decade, with a step increase from 3 to 4 parts per million by volume (p.p.m.v.) in 1988–91, returning to 3 p.p.m.v. in 1992⁶. Although there is

no compelling identification of a common cause, the following general observations can be made. For the period 1988–91 Conway *et al.*⁶ observe an increased equatorial growth rate or increased source (mainly in latitudes 0–30° N), offset by increasing sinks at high latitude. As shown here, the isotopic data require an increased global terrestrial sink during 1988–91, for the location of which the high northern latitudes are the only common possibility. Some support for this comes from the larger isotopic trend anomaly observed at Point Barrow (Figs 1, 2a) but the reason for such increased terrestrial uptake in latitudes 30–90° N is not clear. The increase in the deduced global ocean uptake from 1989 (Fig. 2b) is compatible with the high southern latitude growth-rate decrease. However, the situation is less clear in 1991–92 when the isotopic data imply a further increase in ocean uptake, but Conway *et al.*⁶ prefer an increased high northern terrestrial sink. Ocean uptake associated with cooling due to the 1991 Mt Pinatubo eruption is in this direction but thought to be small⁶. What is clear is that the persistent atmospheric slow-down from 1988 starts with strong increases in both terrestrial and ocean net uptake. □

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Effect of shallow-level contamination on the helium isotope systematics of ocean-island lavas

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THE bimodal distribution of helium isotope ratios in ocean-island lavas has provided powerful constraints on the composition and evolution of the Earth's mantle^{1–3}. 'High-³He hotspot' ratios (³He/⁴He greater than in mid-ocean-ridge basalt, MORB) found on Hawaii⁴ and Iceland⁵ are thought to trace pristine plumes from the deep(er) mantle, whereas 'low-³He hotspot' values (³He/⁴He ≤ MORB value) at Tristan da Cunha⁶ and St Helena⁷ are considered to characterize plumes composed (in part) of recycled oceanic or continental crust. Here we report the observation of both 'high-³He' and 'low-³He' characteristics⁸ in lavas from a single ocean island—Heard Island, in the Indian Ocean. Whereas the high-³He lavas provide unambiguous evidence for the involvement of a deep-seated plume in their genesis, we argue that the low ³He/⁴He ratios in other lavas result from shallow-level contamination by radiogenic helium before eruption. These observations call into question the presumed association between low-³He ratios (at Heard Island and elsewhere) and ancient crustal material recycled back into the mantle.

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Terrestrial helium isotope (³He/⁴He) variations range over three orders of magnitude, owing to the retention of primordial ³He in the Earth's mantle⁹. Thus mantle-derived materials have significantly higher ³He/⁴He values (up to 30R_A in some hot-spots but 8R_A in MORB, where R_A is the atmospheric ³He/⁴He ratio of 1.4 × 10⁻⁶) than helium produced by radioactive decay in the crust (~0.05R_A). The assumption that mantle-derived samples necessarily retain ³He/⁴He features characteristic of their source region has been questioned recently, however, by observations of ³He/⁴He ratios < MORB in (1) mafic phenocrysts of subaerial volcanics^{10–12}, and (2) glassy pillow rims of evolved submarine lavas¹³, and by helium isotope disequilibrium between hydrothermal fluids and basalts¹⁴. These observations have been explained by pre-eruptive (magma chamber) contamination by crustal helium. An important motivation of the present study, therefore, is to test whether ³He/⁴He ratios can provide a reliable indication of mantle source characteristics of ocean islands in light of the fact that most ocean island basalts erupt through thickened lithosphere away from plate margins. Heard Island is well suited for this purpose as its position atop the Kerguelen Plateau increases the potential for crust–mantle interaction¹⁵.

Olivine (ol) and/or clinopyroxene (cpx) phenocrysts were separated from a suite of 16 lavas for ³He/⁴He analysis. The clinopyroxenes are generally euhedral, contain mostly melt and oxide inclusions and vary in size up to 10 mm in diameter. The olivines are smaller (max. ~3 mm) and tend to have a higher proportion of fluid relative to melt inclusions. Of the 16 samples, 11 come from the Big Ben series (BBS) and five from the Laurens Peninsula series (LPS)^{16,17}. Closed-system magma evolution is considered to characterize LPS magmas because phenocrysts are normally zoned and in equilibrium with the melt. In contrast, BBS phenocrysts display complex compositional variations reflecting open-system behaviour involving magma mixing. Current studies of the ²³⁸U/²³⁰Th characteristics of morphologically young surface lava flows (ref. 18 and G.E.W., unpublished data) have consistently found isotopic disequilibrium in samples (*n* = 10) thus limiting their age to <350 kyr. Other samples reported here, occurring lower in the stratigraphic sequence of Big Ben and Mt Dixon (LPS), are also considered young by analogy to K–Ar ages (~130 kyr) of similarly located samples¹⁹.

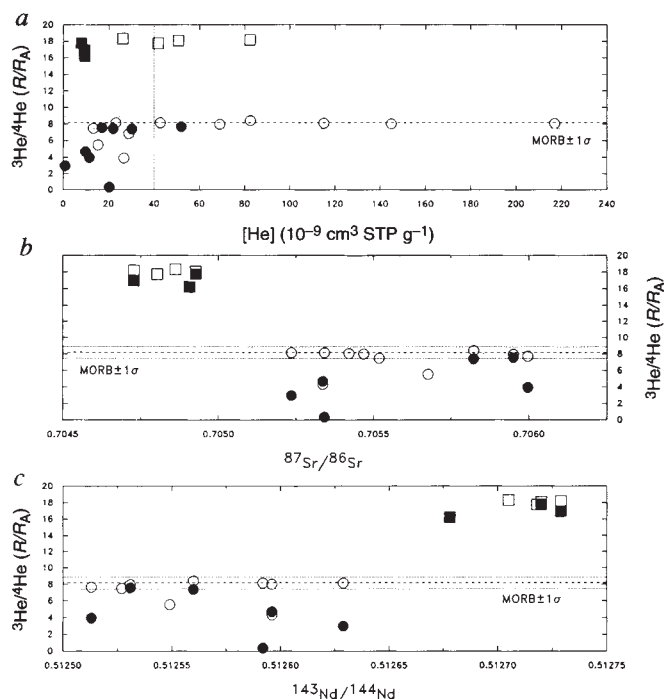


FIG. 1 Plot of $^3\text{He}/^4\text{He}$ ratios (R , normalized to the atmospheric ratio R_A) of Heard Island phenocrysts against: a, total $[\text{He}]$ released by crushing; b, whole-rock $^{87}\text{Sr}/^{86}\text{Sr}$ ratios; and c, whole-rock $^{143}\text{Nd}/^{144}\text{Nd}$. Circles and squares represent Big Ben series (BBS) and Laurens Peninsula series (LPS) lavas respectively, and open and filled symbols are olivines (ols) and clinopyroxenes (cpxs) respectively. Note that high $[\text{He}]$ phenocrysts give the same (MORB-like) $^3\text{He}/^4\text{He}$ ratio for the BBS (a) whereas low $[\text{He}]$ phenocrysts ($<40 \times 10^{-9} \text{ cm}^3 \text{ STP g}^{-1}$) are variably susceptible to contamination by crustal (radiogenic) helium. LPS lavas do not show the same effect, possibly because of closed-system behaviour¹¹ or eruption through younger wall rock devoid of radiogenic He. The plots b and c show the distinction between the two lava suites in Sr and Nd isotopes, and the fact that $^3\text{He}/^4\text{He}$ ratios are almost always lower in cpxs than co-genetic ols. The constancy of the (ol) $^3\text{He}/^4\text{He}$ ratios over a wide range of Sr and Nd values (clearly illustrated for the BBS) indicates 'decoupling' of helium from these other tracers. The MORB $^3\text{He}/^4\text{He}$ ratio is taken from a compilation in ref. 13.

In Table 1 we report $^3\text{He}/^4\text{He}$ analyses of the ol and cpx phenocrysts. The most striking feature of the results is the clear distinction in $^3\text{He}/^4\text{He}$ ratios between the LPS and BBS lavas (Fig. 1). The $^3\text{He}/^4\text{He}$ ratios for the LPS vary within a narrow range ($16.2\text{--}18.3R_A$) and identify Heard Island as a 'high- ^3He ' hotspot. In contrast, BBS phenocrysts are characterized by a wide range in $^3\text{He}/^4\text{He}$ ratios. They vary from $8.4R_A$, as observed in MORB, through the range $5 < R/R_A < 8$ normally associated with helium from 'low- ^3He ' hotspots, to $0.4R_A$ (one sample only—HC26 cpx) which represents predominantly radiogenic helium. Heard Island is, therefore, the first reported occurrence of an ocean island having 'high- ^3He ', MORB-like and 'low- ^3He ' helium isotope characteristics.

There are systematic relationships between $^3\text{He}/^4\text{He}$ ratios, the concentration of helium ($[\text{He}]$) released by crushing and the type of phenocryst analysed. We draw particular attention to three features: (1) the highest $[\text{He}]$ and $^3\text{He}/^4\text{He}$ ratios are found in the ol phases of both lava series. Taking the six highest $[\text{He}]$ olivines of the BBS, their R/R_A values are constant and plot within error of MORB values. Similarly, the four ol samples of the LPS also have $^3\text{He}/^4\text{He}$ ratios within error (mean, $18.1R_A$); (2) in all cases, cpx samples have equal or lower $^3\text{He}/^4\text{He}$ ratios and $[\text{He}]$ than coexisting olivines; and (3) only phenocrysts with low $[\text{He}]$ (cpxs and ols) have $^3\text{He}/^4\text{He}$ ratios more radiogenic than the values defined by the high $[\text{He}]$ olivines.

The inferred young age of the lavas, coupled with the technique of reduced crushing times, minimizes the possibility that deviations from MORB-like helium (BBS) or $18.1R_A$ helium (LPS) result from contamination with (post-eruptive) radiogenic helium. For example, assuming that (cpx) crystals contain 1% melt inclusions which release their volatiles on crushing (and have a uranium content of ~ 1 p.p.m. as in the lavas; G.E.W., unpublished data) then only sample H61 (Table 1) of the BBS needs less than 350 kyr at the surface to grow-in sufficient radiogenic helium to lower the $^3\text{He}/^4\text{He}$ ratio from $8.4R_A$ to its measured value (in contrast, HC26 cpx would require ~ 9 Myr). Alternatively, release by crushing of $\sim 20\%$ of the total matrix-sited (pure) radiogenic helium (calculated from the powder melt data in Table 1) could explain the BBS $^3\text{He}/^4\text{He} < \text{MORB}$. Again, such an effect is highly unlikely given the experimental methodology adopted. We conclude that the principal factor modifying the $^3\text{He}/^4\text{He}$ ratios of some low $[\text{He}]$ samples is pre-eruptive contamination by crustal fluids containing radiogenic helium.

The $^3\text{He}/^4\text{He}$ disequilibrium between ol and cpx offers insights into the circumstances of the contamination. As helium diffuses very rapidly at magma temperatures²⁰, helium isotope equilibrium should characterize co-genetic ols and cpxs. At $1,150^\circ\text{C}$, for example, magma helium should completely exchange with ol crystals (up to 3 mm diameter) in ~ 60 yr, and with cpx (10 mm) within ~ 11 yr. These are maximum estimates as helium is probably more mobile because of internal fractures²⁰. To maintain isotopic disequilibrium, therefore, addition of radiogenic helium must occur at a late stage and immediately before eruption. Altered oceanic crust and/or overlying sediments^{21–23} of the Ker-

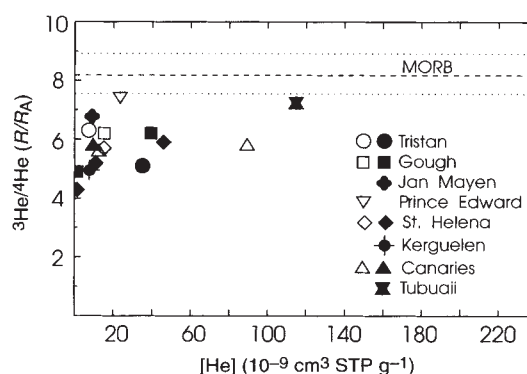


FIG. 2 Plot of phenocryst ol and cpx $^3\text{He}/^4\text{He}$ ratios (R/R_A notation) versus helium concentration ($[\text{He}]$) for low- ^3He hotspots^{6,7,27}. For the most part, the eight islands in the plot have either enriched mantle (EM1 and EM2) or high U/Pb (HIMU) geochemical affinities (see, for example, refs 8 and 28). Only samples processed by vacuum crushing are plotted and only the highest $[\text{He}]$ of replicate analyses (symbols and horizontal scale as in Fig. 1). Note that helium analyses of xenocrystic ol and cpx^{6,27} and evolved lavas^{5,29} are not included because of doubts as to their provenance and extent of crustal interaction respectively. Most samples in the plot have $[\text{He}] < 40 \times 10^{-9} \text{ cm}^3 \text{ STP g}^{-1}$ which seems to be the upper limit for preservation of source $^3\text{He}/^4\text{He}$ ratios of the BBS lavas. Without evidence to the contrary, it is possible that the $^3\text{He}/^4\text{He}$ ratios of these low- ^3He hotspot islands could have been modified before eruption. For example, $^3\text{He}/^4\text{He}$ ratios at the upper end of the MORB range ($9.6R_A$) have been reported recently³⁰ for geothermal fluids from La Palma (Canary Islands) indicating this locality should not be classified as a low- ^3He hotspot. In this respect, it should be noted that the two highest $^3\text{He}/^4\text{He}$ ratios also fall within error of MORB: the sample from Tubuaii (HIMU), which is unlikely to have suffered significant change in $^3\text{He}/^4\text{He}$ because of its high $[\text{He}]$, and the ol of Prince Edward Island (EM) which is less likely to be contaminated than some of the cpxs. Although the true source ratio of these islands may never be known without more (extensive) sampling, the above points indicate that the original $^3\text{He}/^4\text{He}$ ratios of these hotspots could have been MORB-like.

guelen Plateau could be the source of the radiogenic helium, and contamination most likely occurred in shallow magma chambers by trapping of crustal fluids as inclusions in already formed or precipitating crystals. The lower $^3\text{He}/^4\text{He}$ ratios in the cpx probably result as a consequence of the higher He diffusivity in cpx whereby it exchanges more rapidly with crustal fluids or at

a lower temperature when, in effect, the olivines have become closed. Phenocrysts with low mantle-derived [He] would record preferentially the effects of the crustal fluids. Open-system behaviour of magma chambers, as predicted at Big Ben volcano, would result in enhanced pre-eruptive degassing and could contribute to the wider range in [He] in these phenocrysts.

TABLE 1 Phenocryst helium and whole-rock isotope results

Sample	Phase and method*	Wt (mg)	[He] (10 ⁻⁹ cm ³ STP g ⁻¹)	R/R _A †	⁸⁷ Sr/ ⁸⁶ Sr‡	¹⁴³ Nd/ ¹⁴⁴ Nd‡
Big Ben, basalt series						
HC26§	ol(CR)	926	42.7	8.2 ± 0.5	0.705341	0.512592
	ol(PM)	234	14.3	1.5 ± 0.1		
	cpx(CR)	734	20.3	0.37 ± 0.04**		
	cpx(PM)	233	56.2	0.080 ± 0.030		
65143	ol(CR)	471	15.3	5.5 ± 0.2	0.705676	0.512549
	ol(dup)(CR)	491	11.7	5.5 ± 0.4		
	ol(PM)	118	19.6	0.93 ± 0.10		
65151	ol(CR)	1,011	28.8	6.8 ± 0.3	0.705997	0.512513
	ol(dup)(CR)	485	13.3	7.7 ± 0.6		
	ol(PM)	93.8	3.4	2.7 ± 0.2		
	cpx(CR)	808	11.5	4.0 ± 0.2		
65171	cpx(dup)(CR)	890	9.9	3.6 ± 0.2	0.705336	0.512596
	cpx(PM)	134	42.4	0.38 ± 0.04		
	ol(CR)	468	26.8	3.9 ± 0.2		
	ol(dup)(CR)	882	13.6	4.3 ± 0.2		
	ol(PM)	212	19.1	0.52 ± 0.10		
	cpx(CR)	462	9.8	4.7 ± 0.2		
65085	cpx(PM)	173	22.0	0.68 ± 0.05	0.705468	0.512596
	ol(CR)	480	145	8.0 ± 0.4		
	ol(CR)	610	13.2	7.5 ± 0.4		
69264	ol(CR)	628	217	8.1 ± 0.2	0.705420	n.d.
HC29¶	cpx(CR)	504	52.1	7.7 ± 0.2	n.d.	n.d.
	ol(CR)	962	82.6	8.4 ± 0.4		
	cpx(CR)	656	22.0	7.5 ± 0.4		
69261	ol(CR)	968	69.1	8.0 ± 0.2	0.70595	0.512531
	ol(PM)	281	3.4	5.8 ± 0.3		
	cpx(CR)	390	17.0	7.6 ± 0.9		
	cpx(PM)	125	9.3	3.4 ± 0.3		
69284	ol(CR)	840	115	8.1 ± 0.2	0.705822	0.512560
	ol(dup)(CR)	357	124	8.4 ± 0.4		
	cpx(CR)	363	30.2	7.4 ± 0.2		
Big Ben; basanite series						
H61#	ol(CR)	1,025	23.2	8.2 ± 0.5	0.705235	0.512629
	cpx(CR)	1,010	0.94	3.0 ± 0.5		
	cpx(PM)	183	2.5	5.2 ± 0.4		
Laurens Peninsula						
65054	ol(CR)	503	41.8	17.8 ± 0.9	0.704803	0.512718
65254	ol(CR)	373	82.3	18.2 ± 0.3	0.704728	0.512729
	ol(PM)	89.3	1.0	3.2 ± 0.2		
	cpx(CR)	300	9.3	17.0 ± 0.6		
	cpx(PM)	121	2.9	2.3 ± 0.2	0.704907	0.512678
69258	cpx(CR)	703	9.4	16.2 ± 0.4		
69260	ol(CR)	283	50.7	18.1 ± 0.3	0.704927	0.512720
	cpx(CR)	982	8.0	17.8 ± 0.4		
HB24A☆	ol(CR)	345	26.2	18.3 ± 0.9	0.704862	0.512705

* Olivine (ol) and/or clinopyroxene (cpx) phases were crushed *in vacuo* (CR) with released volatiles processed following procedures described previously^{11,14}. Two essential precautionary measures were adopted for the present sample set: (1) reduced crushing times (~60 s) to minimize the possibility of cross-contamination of inclusion helium with matrix-sited helium¹¹ and (2) each phase crushed separately to test for He isotopic disequilibrium between ol and cpx¹¹⁻¹³. Note that all duplicate runs by crushing fall within (2 σ) error. Selected residual powders (from the crushing experiments) were melted (PM) and analysed for He using a VG5400 mass spectrometer at the Vrije Universiteit Amsterdam. Full experimental details will be reported elsewhere. The PM [He] can be resolved into a radiogenic helium component (0.05 R_A) and a mantle component (8.4 R_A , BBS; 18.1 R_A , LPS). We use the PM radiogenic [He] to calculate the percentage which would have to be released during (reduced) crushing to lower the BBS $^3\text{He}/^4\text{He}$ ratios from the MORB value.

† $^3\text{He}/^4\text{He}$ ratios (R) are quoted normalized to the atmospheric $^3\text{He}/^4\text{He}$ ratio ($R_A = 1.4 \times 10^{-6}$). Quoted errors (2 σ) represent mainly measurement statistics on sample and standard ion beams. Neon was monitored in all cases for the crushing experiments and resulted in a minor correction (<5%) in two cases (HC26 cpx, 65143 ol)—all other samples had He/Ne > 1,000. Blank corrections were of the order of $\leq 1\%$ (crushes) $\leq 10\%$ (powder melts); errors on [He] are estimated at $\pm 5\%$ (crushes) and $\pm 10\%$ (powder melts).

‡ From refs 16, 17 and J.B., unpublished data with the following differences: § Sr, Nd from same flow (sample HC27); || 1985 lava flow; ¶ same flow as 69264; #unleached aliquot for Sr and Nd; ☆ Sr, Nd, Pb on sample 69244 from same flow; n.d., not determined.

** This one (cpx) sample is characterized by predominantly radiogenic helium ($^3\text{He}/^4\text{He} < 1R_A$). This may be a consequence of its higher proportion of melt inclusions (>1%) compared to other cpx crystals. Therefore, some release (by crushing) of post-eruptive radiogenic helium could have occurred and its $^3\text{He}/^4\text{He}$ value should be treated with caution.

The distinctive $^3\text{He}/^4\text{He}$ characteristics of LPS ($\sim 18.1R_A$) and BBS ($\sim 8.4R_A$) lavas have a clear bearing on the nature of magma sources supplying Heard Island. First, the high $^3\text{He}/^4\text{He}$ ratios of the LPS lavas provide unambiguous evidence for involvement of a mantle-plume component in their genesis. This is somewhat surprising given the postulated location of the Kerguelen plume beneath prominent seamounts on the north-west margin of the Kerguelen Plateau ~ 550 km from Heard²⁴. Either the LPS lavas represent a fossil remnant of the plume embedded in the thickened lithosphere of the Kerguelen Plateau and reactivated by the voluminous activity of Big Ben volcano¹⁷, or (more speculatively) there are two plumes in this part of the Indian Ocean. Second, there is no helium isotope evidence that the LPS lavas are related to the BBS volcanics by a binary mixing relationship^{16,17}. Indeed, the constancy of the BBS $^3\text{He}/^4\text{He}$ ratios even at relatively radiogenic Sr (or unradiogenic Nd) isotopic compositions (Fig. 1) does not support the suggestion of an ancient 'recycled' crustal component in their genesis, as such a component would be expected to be characterized by radiogenic helium as well as radiogenic strontium. If the source of radiogenic helium in some samples is the Kerguelen lithosphere, as argued above, then a greater role for magma contamination may be necessary to account for various geochemical features of Heard Island lavas.

The realization that shallow-level radiogenic helium can contaminate mantle-derived phenocrysts at Heard Island raises the possibility that other ocean-island $^3\text{He}/^4\text{He}$ ratios (<MORB value) have been similarly affected. In Fig. 2 we plot all low- ^3He hotspots defined by ol and cpx phenocryst $^3\text{He}/^4\text{He}$ ratios. Note that: (1) there are relatively few analyses available from each island; (2) three of the eight islands have been classified by cpx analyses alone; and (3) most phenocrysts have low [He]. We argue that without extensive sampling of different lavas flows to check for constancy of $^3\text{He}/^4\text{He}$ over a range of [He] (as is the present study), then the assumption that the $^3\text{He}/^4\text{He}$ ratios of these samples have not been affected by shallow-level interaction is clearly questionable. For example, if the arbitrary value of $\sim 40 \times 10^{-9} \text{ cm}^3 \text{ STP } ^4\text{He g}^{-1}$ for BBS phenocrysts (Fig. 1a) is generally applicable, then most samples in Fig. 2 are immediately suspect—particularly the cpxs which seem more susceptible to record the influence of shallow-level crustal helium. Without further sampling, the above points cast considerable doubt on the assumed integrity of the low- ^3He phenocrysts, and we suggest that their $^3\text{He}/^4\text{He}$ ratios represent minimum values. We suspect that the source $^3\text{He}/^4\text{He}$ ratio of all these islands is higher and probably close to the MORB value (Fig. 2). In this case, the consequences for the use of helium with other petrogenetic isotopes is profound. The Sr–Nd–Pb isotope systematics of ocean islands lie within a tetrahedron delineated by four mantle endmembers (a depleted MORB mantle component, DMM; and three enriched components—HIMU (high U/Pb) and 'enriched mantle' components EM1 and EM2)^{25,26}. MORB-like $^3\text{He}/^4\text{He}$ ratios at Heard Island (EM1–EM2 characteristics) and possibly at other low- ^3He hotspots (such as St Helena, HIMU) at the opposite apices to the DMM apex of the tetrahedron show that helium can be completely decoupled from Sr–Nd–Pb isotopes. Therefore, helium cannot be used to infer the provenance of 'enriched materials' in mantle plumes. Alternatively, as the (upper) lithosphere can be the source of the radiogenic helium in mantle-derived materials, $^3\text{He}/^4\text{He}$ ratios <MORB may instead identify those samples whose 'enriched' character arises, in part, from lithosphere or crustal interactions. □

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Carnivorous sponges

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EXTREMELY food-poor environments, such as the deep sea, place extraordinary demands on organisms with respect to feeding, resulting in modifications of the feeding strategies found in shallow waters. A general rule is that macrophagy becomes a better strategy than microphagous suspension-feeding^{1–3}. The characteristics by which phyla are defined, nonetheless, remain unchanged in these adaptations. We present here an apparently unique example of a fundamentally different body plan, derived from a pre-existing phylum, occurring in deep-sea sponges. We demonstrate that the Cladorhizidae have evolved carnivory and capture small crustaceans by means of filaments provided with raised hook-shaped spicules. This adaptation to a food-poor deep-sea environment has resulted in the loss of the diagnostic characteristics of the phylum Porifera: an aquiferous system and choanocytes.

Sponges are considered to represent one of the first steps in multicellular organization during early metazoan evolution because of their organizational simplicity and the resemblance of their diagnostic pumping cells, the choanocytes, to protozoan choanoflagellates^{4,6}. Sponges are filter-feeders *par excellence*. Their entire body is organized for filtering water, which is moved inside an aquiferous system by choanocytes. It has long been suspected that the simple filtering system, highly efficient in the retention of particles and colloids in shallow-water environments^{7,8}, becomes modified in deep-sea conditions. Little is known about possible adaptations to low particle concentrations and still-water conditions because it is so difficult to access these remote environments. The discovery of deep-sea sponges in a shallow Mediterranean marine cave, in which conditions closely resemble those of the deep sea^{9,10}, has made it possible to observe for the first time how sponges feed in such extreme environments. We found a new cladorhizid sponge in the cave at a depth of between 17 and 23 m. This species belongs to the

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FIG. 1 Clustered specimens, no more than 15 mm high, of an undescribed species of *Asbestopluma*, the otherwise deepest known genus of Porifera, in a Mediterranean cave at a depth of 18 m. The sponge body is anchored by a spicule peduncle and bears long, thin filaments. Unlike other poriferans, this sponge has neither aperture nor canal system, but feeds mainly on swimming prey captured passively by the filaments.

genus *Asbestopluma*, which holds the depth record for all sponge genera (down to 8,840 m¹¹). The Cladorhizidae are an exclusively deep-sea family of demosponges, most of whose species are localized in the abyssal zone. Some even live in the hadal trenches. Although these sponges are easily classifiable in the order Poecilosclerida on the basis of spicule features, their hydroid-like shape is unusual for sponges.

The anatomy and biology of the new species could be observed both on living and well preserved specimens. It is devoid both of choanocytes and an aquiferous system. Specimens observed *in situ* (Fig. 1) have long, thin filaments. After *in situ* fixation, which induces only slight contraction, no oscula or inhalant ostia are visible (Fig. 2a). The stalked body and the filaments, displaying no canal system or choanocyte chambers, are composed of a loose tissue containing highly ramified stellate cells. Intercellular thread-like bacteria are dispersed in the tissue, and

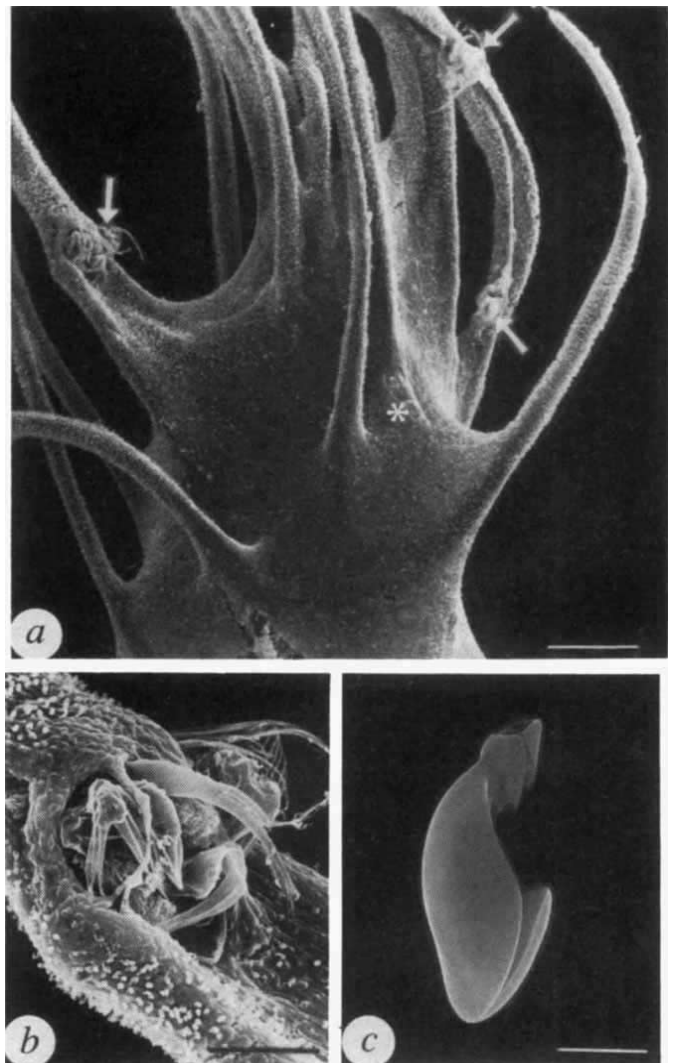
FIG. 2 a, Scanning electron microscope (SEM) view of a specimen of *Asbestopluma* sp. fed with live nauplii of brine shrimp (arrows) 40 h earlier. The body, devoid of aperture, displays a smooth surface, whereas the filaments are covered by raised, hooked spicules (anisochelae). A nauplius is almost completely engulfed within the body (*). Scale bar, 285 μ m. b, Enlarged view of a nauplius trapped by a filament, which sends extensions over the prey (arrow head). Scale bar, 100 μ m. c, SEM view of a microscle spicule (palmate anisochelae). Scale bar, 3.8 μ m.

METHODS. Most feeding experiments were *in situ* because of the extreme fragility of filaments, which entangled when they were exposed to turbulence. About 50 specimens were detached with their rocky support and placed on plastic (PVC) dishes, 10 cm in diameter, wired to grids near the natural population. The sponges were allowed to recover for 3 days. Polyethylene bottles (250 ml) were fitted tightly over the dishes and 25 ml of nutrients or particles were added with a syringe through rubber plugs. For 1 h, sponges were given: fluorescent latex microspheres (Polysciences, 0.1 and 1.1 μ m diameter, 10^6 and 10^7 particles per ml); FITC-labelled dextrans (Sigma, 1×10^3 and 2×10^6 daltons at a concentration of 2 mg l⁻¹); cultures of unicellular algae (*Tetraselmis striata*, 15 μ m; *Agmenellum* sp., 1–2 μ m); and 24-h-old nauplii of the brine shrimp *Artemia salina*. Specimens were also laboratory-raised in a dark room at 13 °C and fed with a cavernicolous mysid crustacean (*Hemimysis speluncola*, 2–6 mm long). Sponges were fixed *in situ* in OsO₄, mercury chloride 6:1 for 90 min.

ovoid bacteria are abundant within bacteriocytes. The surface of the filaments is thickly covered by minute spicules (anisochelae) disposed at right angles to the axis and embedded by the small end in collagen fibrils (Fig. 2b, c). Each anisochela is enclosed in its secreting cell. The spicule cover, absent both from stalk and body surface, gives the filaments a 'Velcro'-like adhesiveness.

How can such a sponge feed in the absence of a filtering system? The dramatic increase in external exchange surface due to the filaments and their dense cover of raised cells supported by the spicules suggests that the animal feeds by surface phagocytosis or osmotrophy. Uptake of dissolved organic matter at least partially fulfils the energy requirements of numerous deep-sea animals, including the gutless Pogonophora¹, and would be possible for the sponge because of its small size, low-density tissue and the increase in surface exchange. The abundance and diversity of symbiotic bacteria might also be an adaptation to an osmotrophic mode of life¹². Another more bizarre hypothesis, first proposed in ref. 13 but later discounted^{14,15}, is that the spicules on the surface of the appendages function as 'claws' to capture minute animals and particles.

In situ tests for the more conventional alternatives, phagotrophy and osmotrophy, were negative. The sponge absorbed neither particles nor large molecules, contrary to the heavy uptake of particles by other sponges, including another deep-sea species living in the same cave, the hexactinellid *Oopsacas minuta*. By contrast, small crustaceans were caught on the non-motile filaments (Fig. 2a, b), which proved to be very efficient in the passive capture of swimming crustaceans, trapping their



thin appendages in the anisochelae. Once captive, the crustaceans were unable to free themselves. They remained struggling for several hours, which indicates that there was no paralysing or toxic secretion. The filament epithelial cells, however, established close contact with the surface of the live prey as early as 1 h after capture. Intense morphogenetic activity involving extensive cell migration occurred after capture. The implicated filaments became shorter and thicker, frequently becoming entangled. New, thin filaments grew over the prey, which was completely enveloped after one day and digested within a few days. After overfeeding in experimental conditions, a complete reorganization occurred, with a normal appearance being resumed only after several days.

Thus all available evidence shows that the sponge is an effective carnivore. The high frequency of crustaceans found still alive or in various stages of decay, both on the surface and within the bodies of specimens collected in natural conditions, suggests that crustaceans smaller than 1 mm are the animal's main source of food in the cave.

Are the adaptations and unusual mode of life observed in this sponge living in a specific sublittoral environment shared by other Cladorhizidae in their deep-sea habitats? Their anatomy and histology are poorly known because of the difficulties in collecting and adequately preserving deep-sea animals. Our unpublished observations of several correctly preserved deep-water cladorhizids did confirm the general absence of canals, apertures and choanocyte chambers that intrigued early observers^{13–15}. Moreover, lateral expansions provided with hook-shaped spicules are common specialized structures in Cladorhizidae. Small animals are frequently found trapped in specimens collected by trawls. It seems likely, therefore, that the entire family Cladorhizidae has developed non-filter-feeding adaptations. Morphological features indicate that the genera *Asbestopuma* and *Cladorhiza* share the same adaptations for the capture of swimming animals with thin appendages; other adaptations may occur in a third genus, *Chondrocladia*¹⁶.

These sponges may have other nutrient sources as well. As yet unpublished observations on a *Cladorhiza* from a cold seep with methane venting, located 4,900 m deep near Barbados, have shown that it is associated with bacteria displaying the morphological characteristics of methanotrophs. Such an association has so far not been described in Porifera, although it has been shown to sustain some deep-sea molluscs and pogonophorans^{17–19}.

Our results raise fundamental questions about the validity of characteristics used to distinguish the phyla of lower invertebrates. A sponge is defined as "a sedentary, filter-feeding metazoan which utilizes a single layer of flagellated cells (choanocytes) to pump a unidirectional water current through its body"²⁰. Except for being sedentary, the cave *Asbestopuma* and presumably all Cladorhizidae lack these basic sponge attributes. In an extreme environment where active filter-feeding has a low yield, cladorhizids have developed a mode of life roughly similar to that of foraminiferans or cnidarians. Their feeding mechanism relies on passive capture of living prey and on transfer of nutrients into the body through intense cell migrations, the analogue of cytoplasmic streaming in foraminiferan pseudopodia. This may be compared to the emergence of macrophagy in abyssal tunicates, also accompanied by a reduction of the filtering system^{2,3} although in Cladorhizidae the result is more extreme, with a main body plan different from Porifera and resembling no other modern anatomical design.

Such a unique body plan would deserve recognition as a distinct phylum, if these animals were not so evidently close relatives of Porifera. Their siliceous spicules show clear similarities to several families of poecilosclerid Demospongiae. The importance of cell mobility and cytological features such as the structure of the external epithelium and the absence of specialized intercellular junctions are also characteristics of Porifera. Given the evidence thus far, we interpret these organisms to be sponges that have deviated from the filtering organization

typical of Porifera and developed adaptations that accommodate totally different feeding habits in the still, nutrient-poor deep-sea environments. □

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Protection and repair of the nigrostriatal dopaminergic system by GDNF *in vivo*

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GLIAL-CELL-LINE-DERIVED neurotrophic factor (GDNF), a recently cloned new member of the transforming growth factor- β superfamily, promotes survival of cultured fetal mesencephalic dopamine neurons¹ and is expressed in the developing striatum^{2,3}. There have, however, been no reports about effects of GDNF *in situ*. We have used the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which produces parkinsonian symptoms in man, to determine whether GDNF might exert protective or regenerative effects *in vivo* in the adult nigrostriatal dopamine system in C57/Bl mice. GDNF injected over the substantia nigra or in striatum before MPTP potentially protects the dopamine system, as shown by numbers of mesencephalic dopamine nerve cell bodies, dopamine nerve terminal densities and dopamine levels. When GDNF is given after MPTP, dopamine levels and fibre densities are significantly restored. In both cases, motor behaviour is increased above normal levels. We conclude that intracerebral GDNF administration exerts both protective and reparative effects on the nigrostriatal dopamine system, which may have implications for the development of new treatment strategies for Parkinson's disease.

The possibility that Parkinson's disease is caused by lack of sufficient 'dopaminotrophic' support and/or the prospect of treating the disease with a neurotrophic factor has led to searches for 'dopaminotrophic' factors. Tissue extracts as well as several known growth factors^{4–10}, including brain-derived neurotrophic

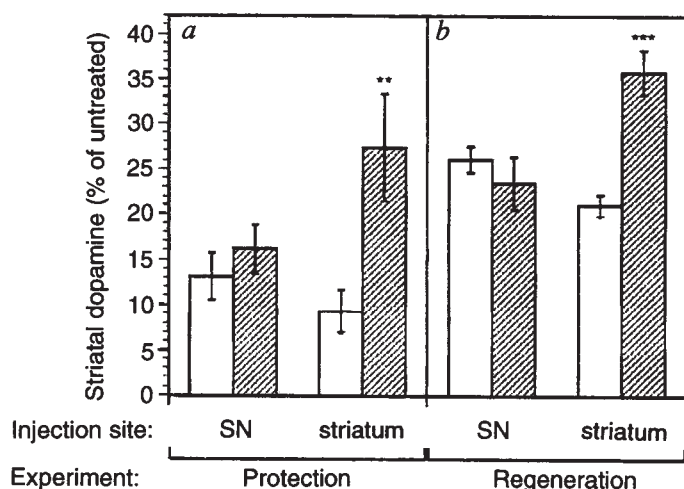


FIG. 1 Striatal dopamine levels in mice given vehicle (open bars) or GDNF (hatched bars) before (a) or after (b) MPTP exposure. Stereotaxic GDNF or vehicle injections were made over the substantia nigra or into the striatum. Dopamine levels $\pm$ s.e.m. in the caudate on the injected side are expressed as percentage of levels in untreated animals. For details of neurochemical measurements, see legend to Table 1. ** $P < 0.01$, *** $P < 0.001$ (ANOVA).

METHODS. C57/BI mice (11–12 weeks) were anaesthetized with halothane, stereotactically injected with 10 μ g GDNF in 2 μ l or 2 μ l vehicle either 24 h before, or 7 days or 7 and 16 days after MPTP (40 mg kg^{-1} for two consecutive days) administration, at the following coordinates: substantia nigra AP: -2.9; L: 1.3, DV: -4.2 mm; striatum: AP: 0.2, L: 2.0, DV: -3.8 mm relative to bregma and the dural surface. Human recombinant GDNF produced in *Escherichia coli* and refolded¹ to full biological activity was used. A stock solution of 40.5 $\mu\text{g } \mu\text{l}^{-1}$ GDNF was diluted in PBS and used for intracerebral injections. Animals were killed and brain areas were rapidly dissected and frozen for monoamine determinations^{13,14}.

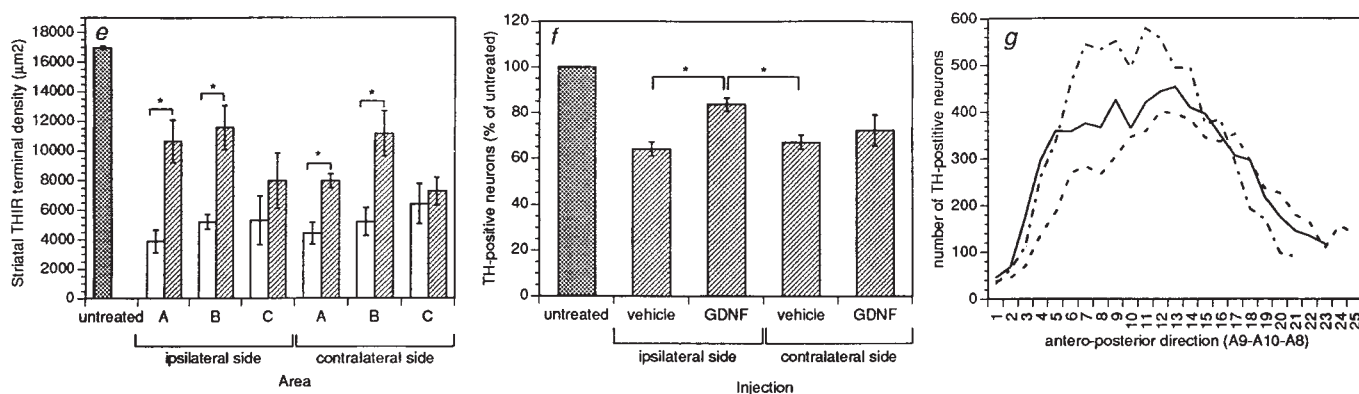
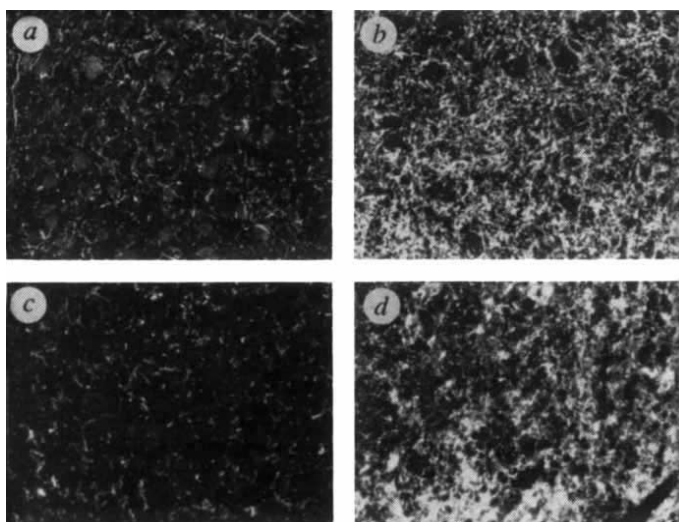


FIG. 2 Tyrosine hydroxylase-positive nerve fibres and cell bodies. a–d, Photomicrographs of TH-IR fibres in 5- μm sections of ipsilateral striatum. Protective experiment: a, Control: MPTP-treated animal given a stereotaxic injection of 2 μ l of vehicle over the substantia nigra. b, Animal pretreated with 10 μ g GDNF over the substantia nigra 24 h before MPTP exposure. Regenerative experiment: c, Control: animals given two stereotaxic injections of 2 μ l of vehicle into the striatum after MPTP treatment. d, Animal given two stereotaxic injections of GDNF into the striatum after MPTP treatment (magnification for a–d: $\times 100$). e, TH-IR fibre density measurements in three areas representing dorsomedial (A), dorsolateral (B) and central (C) parts of the striatum of MPTP-exposed mice, which were pretreated with GDNF (hatched bars) or vehicle injections (white bars). TH terminal density in similarly studied control mice given only saline i.p. was 16,949 μm^2 per unit area (leftmost bar). This density did not show any significant regional variation in untreated mice. f, Dopamine nerve cell counts in 3 animals given GDNF and 3 animals given vehicle injections over the substantia nigra area before MPTP exposure. Twenty-five serial 14- μm sections at 70- μm intervals through the mesencephalic DA cell complex of each brain were used to count all TH-IR nerve cell bodies of groups A8, A9 and A10 separately on the ipsilateral and contralateral sides, generating 490 individual cell group counts. In C57/BI mice, MPTP exposure characteristically causes a loss of DA nerve cell bodies versus control¹⁴. Although cell losses are most marked in substantia nigra pars compacta¹⁴, effects of GDNF treatment were also noted in the other major DA cell groups and were not restricted to the injected side. Average total numbers of TH-IR cells, per level and per animal, were calculated. Normal mice in these experiments contained about 17,148 TH-positive cells in the A8–A10 area (leftmost bar). MPTP treatment leads to a loss of 30% of the TH neurons. f, Total numbers of DA cells. g, Craniocaudal distribution of total numbers of cells per section in untreated mice (dashed and dotted line), GDNF+MPTP treated (solid line) and vehicle+MPTP treated (dashed line) animals.



METHODS. Animals were killed by deep anaesthesia, perfused and further processed for immunohistochemistry using TH-antibodies (Pel-Freezer)². Using a Peltier-cooled CCD camera (Kodak), images were digitized and analysed (MicroGOP Contextvision, SUN Microsystems SparcStation 5 with Sun Video real time video capture and compression subsystem). Density of immunoreactive fibres was calculated averaging three sections per animal. Five animals per group * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, compared with vehicle-treated (control) animals (ANOVA).

TABLE 1 Reverse-phase HPLC data

Group area		DA	HVA	DOPAC	5-HT	5-HIAA	NA
GDNF treatment followed by MPTP							
MPTP s.c. +	SN	473 ± 68	340 ± 57	104 ± 12	1,369 ± 75	971 ± 57	432 ± 38
veh(SN)	CPu	1,783 ± 348	583 ± 94	223 ± 43	277 ± 20	390 ± 37	126 ± 24
n = 13	tub	2,068 ± 187	325 ± 15	311 ± 21	815 ± 66	346 ± 15	111 ± 14
	acc	6,117 ± 259	1,933 ± 970	959 ± 39	1,455 ± 756	806 ± 75	988 ± 161
MPTP s.c. +	SN	785 ± 120**	577 ± 81**	191 ± 29**	1,425 ± 103	1,171 ± 108	603 ± 41**
GDNF(SN)	CPu	2,193 ± 372	918 ± 142	381 ± 42*	322 ± 15	482 ± 39	119 ± 22
n = 13	tub	3,010 ± 697	661 ± 104**	598 ± 141*	955 ± 15	442 ± 22	109 ± 28
	acc	8,618 ± 583	1,646 ± 108	1,162 ± 76	754 ± 10	991 ± 42	1,562 ± 692
MPTP s.c. +	SN	318 ± 21	212 ± 16	88 ± 4	1,450 ± 38	923 ± 103	417 ± 24
veh(CPu)	CPu	1,273 ± 309	539 ± 59	172 ± 24	230 ± 9	370 ± 39	ND
n = 6	tub	2,712 ± 174	375 ± 17	329 ± 22	837 ± 22	320 ± 25	104 ± 15
	acc	4,915 ± 663	926 ± 367	500 ± 71	579 ± 87	586 ± 78	657 ± 222
MPTP s.c. +	SN	914 ± 150**	617 ± 86***	286 ± 49***	1,748 ± 75*	1,195 ± 113	555 ± 54
GDNF(CPu)	CPu	3,720 ± 797**	1,847 ± 280***	768 ± 139***	288 ± 13*	498 ± 38*	67 ± 42
n = 6	tub	3,624 ± 276*	777 ± 78***	723 ± 95***	929 ± 73	438 ± 38**	121 ± 25
	acc	11,338 ± 2733**	2,091 ± 213	1,652 ± 236***	786 ± 117	971 ± 188*	990 ± 238
Controls for protective experiment							
Saline s.c.	SN	645 ± 44	370 ± 14	177 ± 15	1,513 ± 170	817 ± 55	411 ± 35
n = 4	CPu	13,567 ± 1135	1,945 ± 242	956 ± 100	264 ± 11	311 ± 34	167 ± 58
	tub	5,130 ± 387	613 ± 19	565 ± 56	893 ± 61	298 ± 10	112 ± 27
	acc	8,470 ± 976	1,221 ± 162	1,177 ± 208	723 ± 44	662 ± 70	1,397 ± 177
MPTP followed by GDNF treatment							
MPTP s.c. +	SN	558 ± 72	257 ± 43	143 ± 21	1,662 ± 118	798 ± 70	501 ± 34
veh(SN)	CPu	4,656 ± 252	940 ± 58	508 ± 28	348 ± 12	350 ± 13	100 ± 19
n = 5	tub	3,873 ± 208	367 ± 21	352 ± 28	909 ± 41	276 ± 22	158 ± 13
	acc	8,851 ± 977	3,089 ± 574	2,053 ± 324	703 ± 84	1,214 ± 97	1,107 ± 182
MPTP s.c. +	SN	913 ± 126**	350 ± 37*	222 ± 32*	1,906 ± 139	1,024 ± 119	684 ± 63*
GDNF(SN)	CPu	4,172 ± 521	899 ± 58	429 ± 56	392 ± 22	426 ± 12**	83 ± 6
n = 7	tub	4,877 ± 178**	357 ± 21	463 ± 30	999 ± 35	360 ± 19	190 ± 18
	acc	9,448 ± 1207	3,925 ± 702	2,697 ± 226	779 ± 92	1,799 ± 181**	1,253 ± 219
MPTP s.c. +	SN	529 ± 61	258 ± 22	165 ± 18	1,266 ± 225	946 ± 197	453 ± 88
veh(CPu)	CPu	3,765 ± 220	951 ± 48	627 ± 72	317 ± 16	420 ± 16	101 ± 8
n = 8	tub	3,226 ± 193	263 ± 15	492 ± 72	770 ± 40	364 ± 42	242 ± 32
	acc	9,301 ± 1521	3,139 ± 470	1,616 ± 345	640 ± 97	1,098 ± 142	1,271 ± 319
MPTP s.c. +	SN	1,027 ± 379***	463 ± 36***	281 ± 30**	1,879 ± 100**	1,011 ± 51	662 ± 54*
GDNF(CPu)	CPu	6,394 ± 444***	1,441 ± 85***	863 ± 73**	396 ± 38*	447 ± 23	115 ± 10
n = 8	tub	3,849 ± 146	315 ± 25	412 ± 34	888 ± 33*	346 ± 21	193 ± 30
	acc	8,511 ± 1219	2,751 ± 184	1,053 ± 122	794 ± 64	941 ± 47	1,342 ± 284
Controls for reparative experiment							
Saline s.c.	SN	578 ± 109	312 ± 25	169 ± 21	1,364 ± 86	702 ± 18	437 ± 57
n = 5	CPu	17,868 ± 470	1,693 ± 74	1,576 ± 144	333 ± 24	311 ± 21	153 ± 3
	tub	6,457 ± 452	517 ± 44	989 ± 91	791 ± 31	327 ± 29	188 ± 24
	acc	11,655 ± 2371	4,754 ± 2305	2,108 ± 454	859 ± 60	1,432 ± 305	1,741 ± 756

Levels (mean ng g⁻¹ wet weight ± s.e.m.) of dopamine (DA), 5-hydroxytryptamine (5-HT) and noradrenaline (NA), as well as the dopamine metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) and the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the substantia nigra (SN), the caudate-putamen (CPu), tuberculum olfactorium (tub) and nucleus accumbens (acc), as determined by reverse-phase HPLC^{13,14}. In the protection experiments, MPTP treatment led to striatal DA levels which were 13 or 9%, respectively, of levels in saline-treated animals in the two control groups (MPTP + vehicle (veh) into SN, MPTP + vehicle into CPu) 6 days after the first subcutaneous (s.c.) MPTP injection when the mice were 12–13 weeks old. This depletion was paralleled by a marked loss of striatal TH-IR fibres and an increased DA turnover, as indicated by the increased HVA/DA ratio. When GDNF was given instead after MPTP, animals were not killed until 26 days after the first MPTP injection. Basal levels of striatal DA were higher in these 15–16-week-old saline-injected animals than in the 12–13-week-old mice above. DA levels in the two MPTP-treated control groups were 26 and 21% of controls, respectively. The number of TH-IR fibres was markedly reduced and the HVA/DA ratio was roughly doubled. Thus striatal DA levels increase with age in both saline and MPTP-treated mice. While 2 × 40 mg kg⁻¹ MPTP leads to long-lasting DA depletions, a modest degree of recovery appears to occur from day 6 to day 26 after MPTP. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 (ANOVA).

factor (BDNF)⁵, exert variable or modest degrees of dopaminergic activity. However, targeted disruption of the BDNF gene does not lead to any apparent loss of dopamine neurons¹¹. GDNF exerts a trophic influence on grafted fetal mesencephalic dopamine neurons², suggesting that exogenous GDNF could be efficacious *in vivo*. We used the neurotoxin, MPTP, which leads to a well characterized^{12–14} long-term loss of dopaminergic circuitry in C57/Bl mice, to investigate whether pretreatment with GDNF might protect adult dopamine neurons from neurotoxic damage, and furthermore, whether treatment with GDNF after MPTP-induced degeneration might lead to anatomical and functional recovery.

Adult male mice (11–12 weeks, C57/Bl6) were treated with MPTP (40 mg kg⁻¹ subcutaneously (s.c.)) for 2 consecutive days. The mouse strain and age, as well as the treatment regimen, was chosen to lead to marked and long-lasting dopamine depletion in striatum^{14,15}, and a loss of mesencephalic dopamine cell bodies¹⁴. To study if GDNF could protect against the MPTP-induced damage, mice were given unilateral stereotaxic injections of 10 µg GDNF in a volume of 2 µl, or 2 µl vehicle directly above substantia nigra or into striatum 24 h before the first MPTP injection. These animals were killed 7 days after MPTP. To study possible reparative effects, GDNF was given 7, or 7 and 16 days after MPTP using the same intracerebral doses

and coordinates as for the protective protocol. These mice were monitored for 18 days before killing. A total of 174 mice divided into 6 different groups for both the protective and the regenerative experiments were used to study levels of catecholamines, 5-hydroxytryptamine (5-HT) and metabolites, motor behaviour, as well as dopamine cells and nerve fibre densities (protection experiments: biochemistry: $n=38$, histochemistry: $n=51$; regeneration experiments: biochemistry: $n=50$, histochemistry: $n=35$). In addition, control experiments were done to determine normal cell numbers (determined by staining for tyrosine hydroxylase) and nerve fibre densities in untreated mice ($n=4$), the effects of GDNF in normal mice ($n=13$), and the behavioural effects of a higher dose of MPTP ($n=12$).

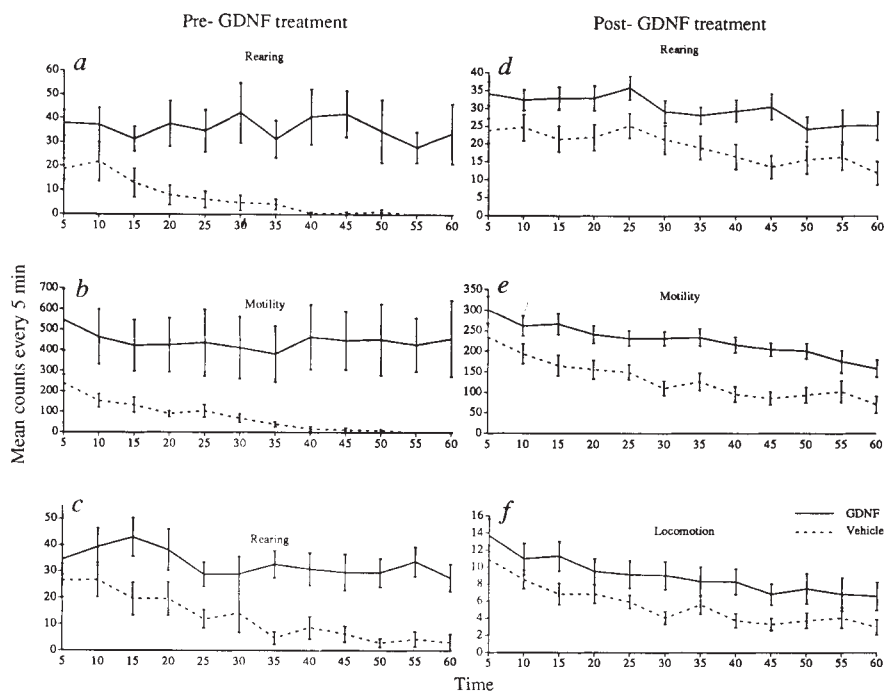
When GDNF was given before MPTP, the dopamine levels were noticeably protected on the side of the intracranial injection both in substantia nigra (Table 1) and striatum (Fig. 1, Table 1) following a striatal injection of GDNF. After a nigral GDNF injection, dopamine levels in the substantia nigra area were partially protected (Table 1), whereas levels in striatum remained low (Fig. 1, Table 1). These protective effects were also reflected in increased levels of the dopamine metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) (Table 1). Smaller increases in 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were also noted, whereas noradrenaline levels appeared less influenced (Table 1). GDNF injected 1 week after MPTP was able partially to restore dopamine levels. These effects were of somewhat smaller magnitude than the protective effects of GDNF, and seen both in substantia nigra and striatum following an intrastriatal injection (Fig. 1, Table 1) but only in substantia nigra following a GDNF injection above the nigra (Table 1). Transmitter level changes in two other areas with a rich dopamine innervation, tuberculum olfactorium and nucleus accumbens, were similar to those found in striatum, although

often of lesser magnitude (Table 1). For both intrastriatal and nigral injections of GDNF, catecholamines and metabolites in contralateral striatal and nigral tissue showed smaller changes in a direction similar to those seen ipsilaterally, particularly in the contralateral substantia nigra after nigral injections (data not shown). Because the nigrostriatal dopamine system is uncrossed, we interpret these effects as due to diffusion of GDNF across the midline following mesencephalic injections. In one set of control experiments GDNF was injected unilaterally into the striatum of normal mice. This treatment did not lead to any significant changes in dopamine levels (data not shown) or dopamine metabolites (data not shown) in striatum or in substantia nigra.

Tyrosine hydroxylase immunohistochemistry was used to visualize catecholaminergic neurons, and monitor GDNF-induced changes at the cellular level. Pretreatment with GDNF led to a marked and highly significant preservation of striatal TH-immunoreactive (IR) nerve terminals on the injected side of the brain (Fig. 2a, b, e). Cell counts revealed a higher number of TH-IR cell bodies in the mesencephalic dopamine cell complex (groups A8–A10¹⁶, including substantia nigra pars compacta) in the GDNF-pretreated mice (Fig. 2f, g). Although effects were noted bilaterally, cell numbers on the GDNF-injected side were significantly higher than on the contralateral side (average side difference per section: GDNF 22.5 ± 15.1 , control 3.3 ± 6.8 ; $P < 0.05$, two-tailed *t*-test). In line with the results from measurements of dopamine levels, the effect of a single injection of GDNF in striatum 1 week after MPTP on TH-IR terminals was less marked. However, when two GDNF injections were given after MPTP, the density of striatal TH-IR nerve fibres was markedly increased (Fig. 2c, d).

Recordings of three measures of motor activity that are thought to reflect basal ganglia dopamine transmission¹⁷ (loco-

FIG. 3 Motor behaviour of mice given intracranial GDNF (solid line) or vehicle (dashed line) injections before or after MPTP exposure. The curves illustrate locomotion, motility and rearing over a 60-min test period from two (protective) or three (regenerative) pooled experiments, each with 4–6 mice per treatment group. Vertical bars indicate s.e.m. at each 5-min time point. a–c, Data from protective experiments studied 6 days after the first MPTP injection; d–f, data from regenerative experiments 11 days after the first MPTP injection. All groups shown were given injections of GDNF or vehicle in striatum, except c, where the injections were placed over substantia nigra. A likelihood ratio test (LRT) was used to find the 'best-fitting' models for the 2 treatment groups simultaneously. The approach used is appropriate for repeated measures data, accounting for variability between mice as well as variability between multiple observations on the same mice. GDNF- and vehicle-injected mice were significantly different in all comparisons (a, b, c, e: $P < 0.001$; d, f: $P < 0.03$). The MPTP dose ($2 \times 40 \text{ mg kg}^{-1}$) was chosen to spare enough of the nigrostriatal DA system to act as a substrate for GDNF in the regeneration experiment. For comparative reasons the same treatment was chosen for the protection experiment. Although this treatment causes marked and highly significant decreases of DA levels and corresponding losses of DA nerve terminals, the remaining DA innervation allows a fairly 'normal' behavioural repertoire in the mice under the present unstressed conditions. Control mice did not differ significantly from the mice given $2 \times 40 \text{ mg kg}^{-1}$ MPTP (data not shown). However, separate control experiments demonstrated that rearing (regarded as the most DA-dependent of these activity measures chosen) was reduced to 27% of control (59 ± 33 , $n=5$ and 217 ± 48 , $n=13$) when the dose of MPTP was increased to $2 \times 50 \text{ mg kg}^{-1}$.



METHODS. Twelve locomotor cages equipped with an array of both horizontal and vertical photocell detectors were used. Locomotion, motility and rearing (vertical activity) were simultaneously recorded by a computerized system using infrared photocells to detect the movements of the mice¹⁷. Motility is defined as a movement covering a distance of 4 cm in any direction, and locomotion is defined as a movement covering 8 photocells or 32 cm. Photocells located 9 cm over the cage floor detected the number of times the mouse stood on its hindlimbs (rearing).

motion, rearing and motility) showed that GDNF given either before or after MPTP was able to significantly increase motor activity scores as compared to animals treated with MPTP and given vehicle intracranially. Indeed, these behaviours were significantly incremented over levels seen in untreated mice. Figure 3 illustrates increases in motor performance parameters elicited by a single dose of GDNF given before (*a-c*) or after (*d-f*) MPTP; Fig. 3c shows data from mice injected over the substantia nigra. All other plots are from intrastriatal injections.

The present results provide the first evidence, to our knowledge, that GDNF can effectively protect midbrain dopamine circuits from the toxic effects of MPTP *in vivo* and also exert a normalizing, regenerative effect when given after MPTP. We have provided biochemical, histochemical and behavioural evidence consistent with a positive role for GDNF in adult dopamine neurons. The observation that better effects were found in the protective experiments when GDNF was injected in striatum, and that in the regenerative experiment GDNF injected over the nigra area was unable to induce sprouting in striatum, suggests that GDNF might act as a typical target-derived factor. Thus, if GDNF is normally synthesized in striatum one should predict that exogenous GDNF would be more potent when injected into the vicinity of the striatal dopamine nerve terminals. The biochemical, histochemical and behavioural changes induced by GDNF correlate well in all protocols, except for the effects of nigral injections in the protective experiment. In this situation, motor activity and striatal TH-IR are increased, despite no significant increment in striatal dopamine levels. The mechanism here may be analogous to our preliminary studies in non-lesioned rats, where unilateral nigral injections of GDNF elicit a twofold increase in striatal TH-IR and a threefold increase in striatal dopamine turnover, but no significant changes in striatal dopamine levels¹⁸. Levels of GDNF mRNA in striatum are apparently not regulated by the nigrostriatal dopamine input because no increase is found after 6-hydroxydopamine-induced

denervation². Instead, GDNF mRNA levels in striatum might be regulated by the excitatory cortical input¹⁹. A similar relationship has been suggested for glutamatergic inputs to hippocampus²⁰. GDNF mRNA is also found in other areas of the developing rodent such as thalamus, the cerebellar anlage, the cingulate cortex, the pineal gland, neurons of Clark's column in the spinal cord² and adult olfactory tubercle. Although the presence of GDNF synthesis in areas outside the nigrostriatal system suggests additional roles for this new trophic factor in the central nervous system²¹, these experiments have demonstrated that local intracerebral treatment with GDNF can offer significant protection of the nigrostriatal dopamine system from the deleterious effects of the specific neurotoxin MPTP and even induce regeneration of dopamine nerve terminals in adult individuals after MPTP treatment. □

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Mesencephalic dopaminergic neurons protected by GDNF from axotomy-induced degeneration in the adult brain

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GLIAL-CELL-LINE-DERIVED neurotrophic factor (GDNF) promotes survival of embryonic dopaminergic neurons in culture¹, and its expression pattern suggests a role as a transient target-derived trophic factor for dopaminergic neurons of the substantia nigra²⁻⁴. These neurons participate in the control of motor activity, emotional status and cognition⁵, and they degenerate in Parkinson's disease for unknown reasons. To test whether GDNF has a trophic effect on dopaminergic neurons in the adult brain, we used a rat model in which these neurons are induced to degenerate by transecting their axons within the medial forebrain bundle⁶. We report here that axotomy resulted in loss of half the tyrosine hydroxylase-expressing neurons in the substantia nigra. This loss was largely prevented by repeated injections of GDNF adjacent to the substantia nigra. Our findings suggest that GDNF or related molecules may be useful for the treatment of Parkinson's disease.

Rat recombinant (rr) GDNF was produced as described¹ and its activity was tested in embryonic mesencephalic cultures enriched for dopaminergic neurons⁷ (Fig. 1a). Fourteen days after medial forebrain bundle (MFB) transection the number of neurons immunopositive for tyrosine hydroxylase (TH), the transmitter-specific marker of dopaminergic neurons, was reduced in the substantia nigra to 52.6% of the unlesioned contralateral side (Figs 1b, 2 and Table 1). In contrast, rats receiving daily injections of rrGDNF (60 µg total) showed survival of 84.6% of dopaminergic neurons (Figs 1b, 2, Table 1). Unlesioned rats which were injected identically to MFB-lesioned rats through implanted cannulas did not show a difference in the number of TH-immunopositive neurons detected on both sides (Table 1). The size of mesencephalic TH-immunopositive cell bodies was unaffected by rrGDNF administration (cytochrome-*c*-injected rats, control side: $90.3 \pm 6.1 \mu\text{m}^2$, lesioned side: $89.7 \pm 3.5 \mu\text{m}^2$; rrGDNF-injected rats, control side: $89.1 \pm 3.7 \mu\text{m}^2$, lesioned side: $88.2 \pm 4.5 \mu\text{m}^2$; means $\pm$ s.e.m. of 20 randomly selected TH-positive neurons in the A9 area from each of 8 rats injected with cytochrome *c* and rrGDNF; analysed using a computerized image analysis system) and there were no changes in TH-immunostaining intensity. The absence of any changes in these parameters might be due to the fact that 14 days after the MFB lesions the axotomy-induced degeneration of nigral dopaminergic neurons is completed⁸.

Analysis of the anteroposterior distribution of dopaminergic neurons in the mesencephalon showed that rrGDNF protected dopaminergic neurons located at different distances from the axotomy to a similar degree (Fig. 1b). Less than maximal protection was observed close to the injection site, probably reflecting nonspecific damage of dopaminergic neurons close to the tip of

the injection cannula. Comparison of cell counts of mesencephalic dopaminergic neurons in the substantia nigra (A8 and A9) and the ventral tegmental area (A10) revealed that rrGDNF protects both groups of dopaminergic neurons to the same extent (Table 1).

Dose-response analysis of the protective action of rrGDNF revealed that daily administration of 5 µg and 1 µg for 14 days was equally effective, whereas 0.2 µg per day resulted only in a partial effect (Table 2a). Dopaminergic neurons in the ventral tegmental area responded to rrGDNF in a way very similar to those of the substantia nigra to the various doses. To evaluate the specificity of the rrGDNF action we also tested neurotrophin (NT)-4/5, transforming growth factor- α (TGF- α) and basic fibroblast growth factor (bFGF) in MFB lesioned rats. These factors had been shown previously to exert trophic activity on dopaminergic neurons⁷⁻¹¹. NT-4/5 and TGF- α were injected at 1 µg per day and bFGF was administered at 10 ng per day based on earlier findings that demonstrated toxic effects at higher dosages *in vivo*¹². Under these conditions, none of the three factors

TABLE 1 Numbers of mesencephalic TH-positive neurons in substantia nigra (A8 + A9) and ventral tegmental area (A10)

Area		Control side	Lesioned side	Lesioned/control (%)
A8 + A9	Control (8)	6,397 ± 542	3,385 ± 530	52.6 ± 5.7
	GDNF (9)	6,630 ± 767	5,469 ± 753*	84.6 ± 7.5**
A10	Control (8)	4,748 ± 354	2,580 ± 137	56.7 ± 5.6
	GDNF (9)	4,602 ± 307	3,729 ± 349**	80.3 ± 4.1**
Total	Control (8)	11,145 ± 896	5,965 ± 667	53.5 ± 5.6
	GDNF (9)	11,232 ± 1,074	9,198 ± 1,102**	81.9 ± 6.2**
Unlesioned rats				
	Control (4)	12,110 ± 796	11,887 ± 963	98.0 ± 2.3
	GDNF (5)	11,377 ± 502	11,251 ± 440	99.0 ± 2.0

Lesions, injections and TH-positive cell counts were done as described in Figs 1 and 2. Unlesioned rats underwent cannula implantations and were injected in the same way as animals in the MFB lesioned groups. Data are means ± s.e.m. from two independent experiments using two batches of rrGDNF which were prepared separately. The total numbers of rats in each group are indicated in brackets. Asterisks indicate significant differences to the lesioned side of the corresponding control group; *, $P < 0.05$; **, $P < 0.01$ (Student's *t*-test).

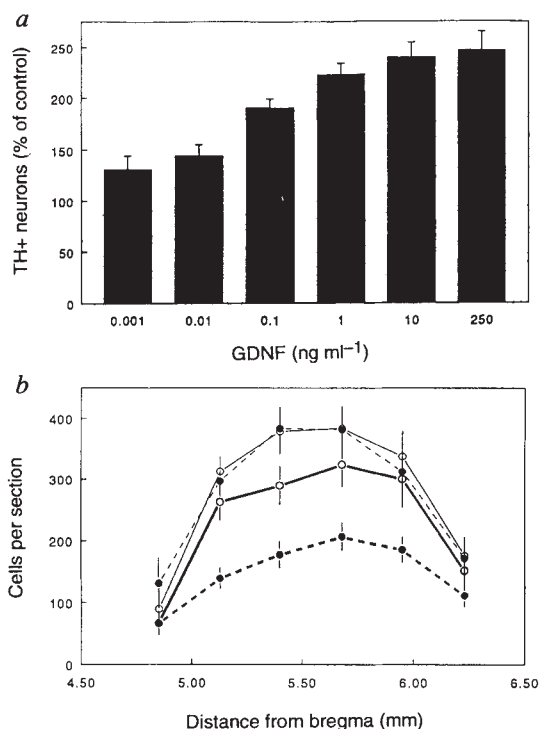


FIG. 1 Trophic actions of rrGDNF on dopaminergic neurons. a, Increase of TH-positive neuron numbers in enriched mesencephalic cultures treated with rrGDNF. Dose-response analysis after 6 days in culture. b, Anteroposterior distribution of TH-immunopositive neurons in the ventral mesencephalon of MFB lesioned rats after 14 daily injections of rrGDNF or cytochrome c (shown are means ± s.e.m. from two independent experiments, $n = 9$ for rrGDNF-, $n = 8$ for cytochrome c-injected rats). Filled circles, cytochrome-c-treated rats; thin dashed lines, unlesioned side; thick dashed lines, lesioned side. Open circles, GDNF-treated rats; thin solid lines, unlesioned side; thick solid lines, lesioned side.

METHODS. Embryonic rat (E14) mesencephalic cultures enriched for dopaminergic neurons⁷ were grown with different concentrations of rrGDNF for 6 days. Medium containing rrGDNF was replaced every day. Dopaminergic neurons were identified by TH immunohistochemistry. rrGDNF was injected in adult MFB lesioned rats as described in Fig. 2. TH-positive neurons were counted by a blinded investigator at high magnification ($\times 100$) in every sixth serial frontal section covering the entire anteroposterior extent of the substantia nigra and ventral tegmental area. The total number of dopaminergic neurons in the two areas was estimated by multiplication of the cell count results with the correction factor of six.

prevented the loss of nigral TH-immunopositive neurons (Table 2b). The loss of dopaminergic neurons in the ventral tegmental area was also unaffected (data not shown). In addition, a previous study revealed that also brain-derived neurotrophic factor (BDNF) at a dose of 0.224 µg per day for 18 days is ineffective in our lesion model⁶. On the basis of our data we cannot exclude that administration of much higher dosages of NT-4/5, BDNF or TGF- α might also protect, at least partially, axotomized dopaminergic neurons. In a similar lesion model, such partial protection of retrogradely labelled nigral neurons with strongly reduced TH-immunostaining intensity after administration of 30 µg per day BDNF for 2 weeks has been reported recently¹³. However, our findings show that GDNF at far lower dosages can protect the majority of axotomized mesencephalic dopaminergic neurons with an intensity of TH-immunoreactivity indistinguishable from unlesioned controls.

Models of axotomy-induced degeneration are instrumental in the search for trophic factors as potential therapeutic agents in neurodegenerative diseases. Our MFB transection model was designed by analogy to axotomy models used before to identify trophic factors successfully for specific neuron populations: NGF prevents degeneration of forebrain cholinergic neurons after fimbria-fornix transection; ciliary neurotrophic factor (CNTF), insulin-like growth factor I (IGF-I), leukaemia-inhibiting factor (LIF), basic fibroblast growth factor (bFGF), BDNF, neurotrophin-3 (NT-3) and NT-4/5, with different efficacy and specificity, prevent degeneration of axotomized motor neurons in spinal cord and cranial nerve nuclei¹⁴⁻²¹; CNTF rescues thalamocortical projection neurons²²; bFGF protects neurons in the entorhinal cortex after perforant path transection²³. Mesencephalic dopaminergic neurons progressively die after axotomy as shown by the loss of nigral cells retrogradely labelled from the striatum at 14 days after axotomy⁸. There is no spontaneous regeneration of axotomized dopaminergic neurons, in contrast to the gradual restoration of dopaminergic function after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration.

Our findings show that GDNF can prevent the degeneration of dopaminergic neurons in the adult mesencephalon, suggesting a potential usage of GDNF in therapeutic approaches to Parkinson's disease and Parkinsonism. GDNF so far is the only identified factor with highly potent trophic activity on dopaminergic neurons *in vivo*. CNTF rescues retrogradely labelled nigral neurons from axotomy-induced degeneration⁸. However, in contrast to GDNF, CNTF failed to protect the dopaminergic phenotype. The rescued retrogradely labelled cells did not express TH, the rate-limiting enzyme in dopamine synthesis.

The physiological function of GDNF in the adult brain is still unclear. GDNF messenger RNA has not been detected in the

TABLE 2 Numbers of mesencephalic TH-positive neurons in substantia nigra (A8 + A9) of rats treated with different dosages of GDNF; comparison with TGF α , bFGF and NT-4/5 treatment

Treatment	Control side	Lesioned side	Lesioned/control (%)
(a) GDNF dose response			
Control (5)	6,450 $\pm$ 484	3,078 $\pm$ 277	47.7 $\pm$ 2.6
5.0 μ g per day (5)	5,790 $\pm$ 162	4,866 $\pm$ 411**	84.1 $\pm$ 6.9**
1.0 μ g per day (5)	5,753 $\pm$ 395	4,650 $\pm$ 228**	81.6 $\pm$ 3.7**
0.2 μ g per day (4)	6,243 $\pm$ 177	3,972 $\pm$ 568	63.0 $\pm$ 7.0*
(b) Specificity			
Control (17)	5,982 $\pm$ 150	3,450 $\pm$ 192	57.7 $\pm$ 3.8
TGF α , 1 μ g per day (7)	5,994 $\pm$ 132	3,690 $\pm$ 162	61.6 $\pm$ 2.7
bFGF, 10 ng per day (4)	5,868 $\pm$ 234	3,984 $\pm$ 194	65.9 $\pm$ 4.3
NT-4/5, 1 μ g per day (5)	4,992 $\pm$ 408	3,138 $\pm$ 312	62.9 $\pm$ 4.9

Lesions, injections and counting of TH-positive cell were done as described in Figs 1 and 2. Data are means $\pm$ s.e.m. from independent experiments done with each trophic factor and cytochrome c controls. The total numbers of rats in each group are indicated in brackets. Asterisks indicate significant differences to the lesioned side of the corresponding control group; *, $P < 0.05$; **, $P < 0.01$ (Student's *t*-test).

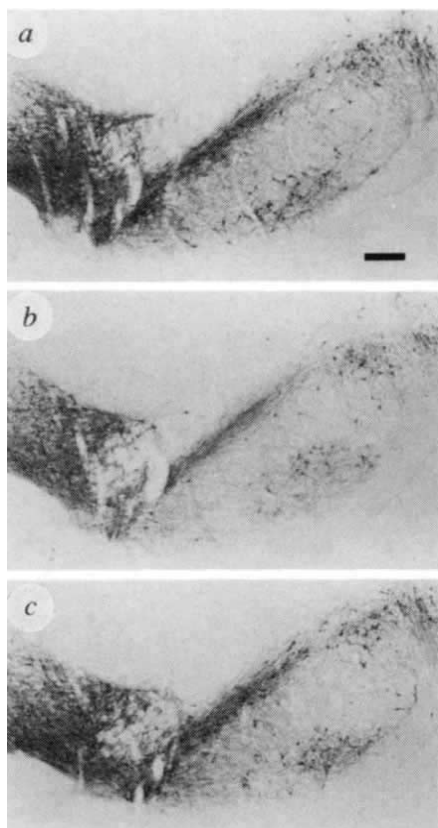


FIG. 2 Mesencephalic dopaminergic neurons visualized by TH immunohistochemistry 14 days after MFB transection. a, Unlesioned control; b, lesioned side after 14 daily injections of control protein; c, lesioned side after 14 daily injections of 4 μ g rrGDNF.

METHODS. A retractable wire knife (k) was used to transect dopaminergic axons ascending in the MFB of adult Wistar rats (220–250 g) as described previously⁶. For repeated injections of rrGDNF or a control protein (cytochrome c) a cannula system (Plastics One) was implanted with the tip located dorsal to the ipsilateral substantia nigra and ventral tegmental area at 5.2 mm posterior, 1.7 mm lateral and 6.0 mm below bregma coordinates. rrGDNF was expressed in *Escherichia coli* and purified as described previously¹. Injections of rrGDNF or cytochrome c (2 μ g μ l⁻¹ in 0.9% saline with 10 mg ml⁻¹ gentamicin) were done daily in a volume of 2 μ l over a period of 2 min. After 14 days, rats were anaesthetized and perfused with phosphate-buffered 4% paraformaldehyde. Histological techniques and TH immunohistochemistry with monoclonal anti-TH antibodies (Chemicon) were described previously⁶.

adult brain under control conditions^{2–4}. However, after pilocarpin-induced status epilepticus GDNF mRNA is detectable in the adult striatum, hippocampus and cortex²⁴, but it is not induced by striatal hydroxydopamine injections which remove dopaminergic terminals³. Alternatively, the sensitivity or expression of GDNF receptors might be affected by lesions of dopaminergic neurons, but these receptors remain to be identified. Although the exact mechanism of action remains to be elucidated, our findings strongly suggest an important role for GDNF in the development of therapeutic approaches for the treatment of Parkinson's disease and Parkinsonism. □

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In vivo neurotrophic effects of GDNF on neonatal and adult facial motor neurons

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MOTOR neurons require neurotrophic factor(s) for their survival during development and for maintenance of function in adulthood^{1–3}. *In vivo* studies have shown that motor neurons respond to a variety of molecules, including ciliary neurotrophic factor, members of the neurotrophin family, and the insulin growth factor IGF-1 (refs 3–13). Here we investigate the potential motor neuron neurotrophic effects of glial-cell-line-derived neurotrophic factor (GDNF), initially identified as a neurotrophic factor for substantia nigra dopaminergic neurons¹⁴. We find that GDNF is retrogradely transported, in a receptor-mediated fashion, by spinal cord motor neurons in neonatal rats. Local application of GDNF to the transected facial nerve prevents the massive motor neuron cell death and atrophy that normally follows axotomy in the neonatal period. In adult rats, GDNF administered locally or systemically can markedly attenuate the lesion-induced decrease of choline acetyltransferase immunoreactivity in the facial nucleus. Our data indicate that GDNF has very profound neurotrophic effects *in vivo* on developing as well as on adult motor neurons, and is the most potent motor neuron trophic factor found so far.

We first examined the ability of spinal motor neurons to transport GDNF in neonatal rats. The retrograde transport of ^{125}I -labelled GDNF was examined by emulsion autoradiography of lumbar spinal cord sections. Dense accumulation of silver grains was found over motor neurons in the ipsilateral spinal motor column (Fig. 1a, c). No silver grains above background were observed in the contralateral spinal cord. Coinjection of a 111-fold excess of unlabelled GDNF completely blocked transport of ^{125}I -labelled GDNF in the ipsilateral side, which indicated that the retrograde transport of GDNF was saturable and receptor-mediated (Fig. 1b, d). The retrograde transport of GDNF was also examined by direct measurement of transported radioactivity in dissected ipsilateral or contralateral half lumbar spinal cords. Consistent with the autoradiographic study, specific transport was demonstrated by the much higher accumulation of radioactivity in ipsilateral spinal cords; this could be completely blocked by coinjection of an excess of 111-fold unlabelled GDNF (Fig. 1e). These results show that motor neurons can bind, internalize and retrogradely transport GDNF in a specific, receptor-mediated fashion.

The demonstration of specific retrograde transport of GDNF suggested that this molecule might have biological effects on motor neurons. Rapid and reproducible motor neuron cell death occurs after axotomy in neonatal rats and ~90% of facial motor neurons degenerate 7 days after axotomy on the day of birth^{9,10}. This experimental protocol was used to assess the protective effects of GDNF on facial motor neurons. The loss of motor neurons after facial nerve transection in control animals treated with cytochrome *c* is apparent (Fig. 2a, c). By contrast, in GDNF-treated animals, all motor neurons survived axotomy over the 7-day period of the experiment (Fig. 2b, e). Cell counts from control and experimental animals showed that less than 6% of motor neurons remained after axotomy in cytochrome-*c*-treated animals compared to almost 100% of motor neurons in animals that received GDNF (Table 1). These data show that GDNF completely prevents the death of motor neurons which normally occurs after axotomy at this age in rats. In addition,

TABLE 1 Motor neuron counts of the facial nuclei of 7-day-old rats after right facial nerve transection on postnatal day 0

Factor	Motor neuron counts $\pm$ s.e.m.		Mean of survival (R/L $\pm$ s.e.m.) $\times$ %
	Transected (R)	Control (L)	
Cytochrome <i>c</i> (<i>n</i> = 6)	230 $\pm$ 36*	3,958 $\pm$ 188	5.87 $\pm$ 0.95
GDNF (<i>n</i> = 5)	4,088 $\pm$ 189	4,112 $\pm$ 106	99.21 $\pm$ 2.16†

Control (L) is the non-lesioned left side. Cells in the facial nucleus with abundant cytoplasm and clear nuclei and nucleoli were blindly counted in every tenth section. Cell counts were not corrected for split nucleoli.

* Axotomy caused a significant degree of cell death in the cytochrome-*c*-treated animals ($P < 0.0001$; paired 2-tail Student *t*-test; right versus left) which was blocked by GDNF treatment.

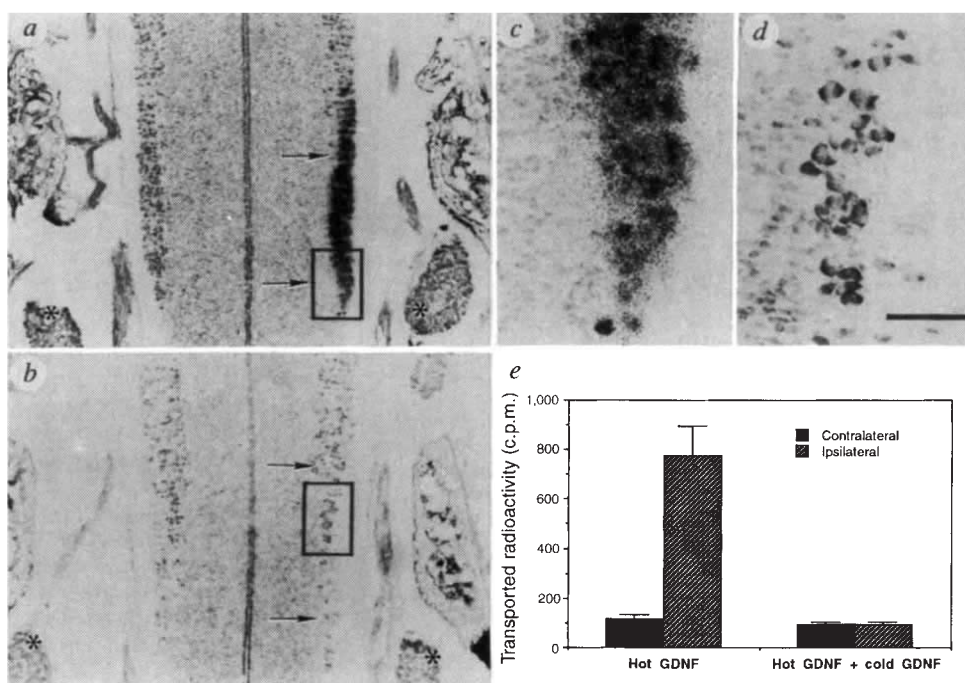
† Value is significantly different from cytochrome *c*-treated animals ($P < 0.0001$; unpaired 2-tailed Student *t*-test).

the GDNF-treated lesioned motor neurons (Fig. 2e) appeared to maintain the normal soma size when compared with motor neurons on the non-lesioned side (Fig. 2d). By contrast, surviving axotomized motor neurons in cytochrome-*c*-treated animals were markedly atrophic (Fig. 2c). This phenomenon was further quantified by morphometric analysis. Motor neurons with abundant cytoplasm and clear nuclei and nucleoli were randomly selected and their sizes measured by tracing cell somata with a Quantimet 520 image analyser coupled to a Nikon microscope. Axotomy caused a significant decrease in the motor neuron soma size in cytochrome-*c*-treated animals (395.3 ± 11.0 after lesion compared to 575.7 ± 14.9 for non-lesion control; $P < 0.0001$, unpaired, 2-tailed Student *t*-test), and GDNF treatment completely prevented this axotomy-induced atrophy (574.3 ± 13.4 after lesion compared to 566.5 ± 14.1 for non-lesion control). Thus, GDNF can effectively prevent both cell death and atrophy of neonatal motor neurons induced by axotomy.

Next we tested whether adult motor neurons were responsive to GDNF. Although adult motor neurons can survive after

FIG. 1 GDNF is retrogradely transported by motor neurons. Bright-field views of autoradiographs of lumbar spinal cords (horizontal section) labelled after injection of ^{125}I -GDNF into the right hindleg footpad. Dense accumulation of silver grains was seen over motor neurons in the ipsilateral (right) spinal cords of animals injected with ^{125}I -GDNF alone (a; and c, a higher-power view of a) but no transport was observed in animals coinjected with a 111-fold excess of unlabelled GDNF (b; and d, higher-power view of b). Arrowheads point to the L4-5 spinal cord and stars indicate L2 DRG. The bar in d represents 500 μm for a and b, 100 μm for c and d. Quantification of retrograde transport of GDNF is shown in e. Solid bars represent contralateral and hatched bars represent ipsilateral half of lumbar spinal cord relative to the side of injection. Values are mean ($n = 7$) $\pm$ s.e.m.

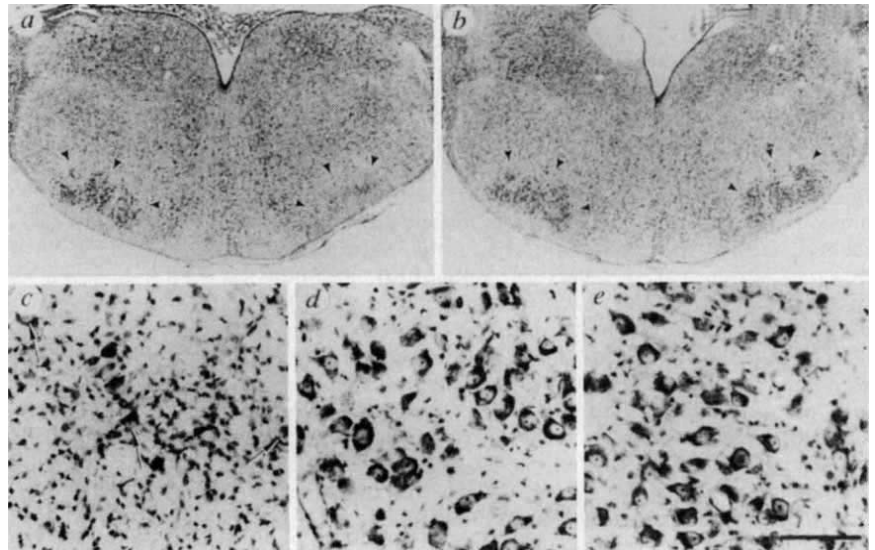
METHODS. Recombinant human GDNF was produced and purified as previously described¹⁴ and iodinated by the lactoperoxidase method²⁰. The iodinated protein was separated from free ^{125}I by G-25 Sephadex quick-spin columns (Boehringer Mannheim). A total of 4.2×10^6 c.p.m. (39.7 ng) of ^{125}I -GDNF alone or with 111-fold excess of unlabelled GDNF in 2 μl PBS were injected into the foot pads of the right hindlegs of 1-day-old



rats. Eighteen hours after injection, animals were killed and either perfusion-fixed for autoradiography ($n = 3$) or had their spinal cords removed for counting in a γ -counter ($n = 7$) as before¹⁰.

FIG. 2 GDNF prevents axotomy-induced motor neuron death in neonatal rats. Transverse sections of brainstem from 7-day-old rats received cytochrome c treatment (a) and GDNF treatment (b) after right facial nerve section on postnatal day 0. High-power views taken from lesioned facial nucleus of cytochrome c-treated rat (c) and the non-lesioned facial nucleus (d) and lesioned facial nucleus (e) of GDNF treated rat are shown. In cytochrome c-treated rats, the few remaining motor neurons on the lesioned side were atrophic (arrows). In contrast, all the lesioned motor neurons in GDNF-treated rats were rescued. In addition, GDNF treatment blocked axotomy-induced motor neuron atrophy. Scale bar in e represents 1 mm in a and b, 100 μ m in c, d and e.

METHODS. The right facial nerves of newborn rats (postnatal day 0) were transected near the stylo-mastoid foramen and a $2 \times 2 \times 2$ (mm³) Gelfoam soaked with 1 mg ml⁻¹ GDNF in PBS was implanted into the cut site. Control animals received cytochrome c (1 mg ml⁻¹ in PBS) soaked Gelfoam. Animals were killed on postnatal day 7, followed by perfusion-fixation with 4% paraformaldehyde. Brainstems were embedded in paraffin, cut into 5- μ m sections and stained with toluidine blue.



axotomy, such a lesion induces a rapid and reproducible decrease of acetylcholine transferase (AChT) immunoreactivity in motor neurons^{3,15}. Seven days after transection of the right facial nerve; AChT immunoreactivity was greatly decreased in the lesioned facial nuclei receiving PBS treatment (Fig. 3). GDNF applied locally to the proximal stump of the cut facial nerve attenuated the lesion-induced decrease of AChT immunoreactivity in facial nuclei. Daily subcutaneously administered GDNF could also attenuate the axotomy-induced decrease of AChT immunoreactivity in a dose-dependent fashion over the 7-day period (Fig. 3). The subcutaneous GDNF treatment over the 7-day period did not change AChT immunoreactivity of non-lesioned facial nuclei and did not affect the soma sizes of either the lesioned or non-lesioned adult facial motor neurons (data not shown).

Our results demonstrate that motor neurons in newborn rats can transport GDNF, and that GDNF has a dramatic survival-promoting effect on neonatal motor neurons in an axotomy-induced cell death paradigm. In addition, axotomized adult motor neurons were responsive to GDNF. The specific retrograde transport of GDNF by motor neurons indicates that motor neurons express a GDNF receptor and can bind and transport GDNF from their target fields to the cell bodies. If GDNF plays a physiological role as a target-derived neurotrophic factor for motor neurons, this factor should be expressed in muscle. In fact, GDNF messenger RNA is expressed in E15 rat limb bud and in cultured neonatal embryonic myotubes¹⁶, suggesting that muscle-produced GDNF might act as a target-derived neurotrophic factor for motor neurons. In addition, GDNF mRNA is made by Schwann cells in the periphery¹⁶ and by type 1 astrocytes in the central nervous system¹⁷. Thus GDNF may also act on motor neurons in a non-target-derived fashion as well as on other populations of non-dopaminergic neurons¹⁸.

We found that not only spinal motor neurons but also neonatal dorsal root ganglia neurons could retrogradely transport GDNF (data not shown); we are currently investigating whether GDNF acts as a neurotrophic factor for sensory neurons. In contrast to neonatal motor neurons and sensory neurons, we found no retrograde transport of GDNF by superior cervical ganglion (sympathetic) after injection into the anterior chamber of the eye of adult rats (data not shown). The failure of retrograde transport of GDNF by adult sympathetic neurons and the lack of response of cultured sympathetic neurons to GDNF¹⁶

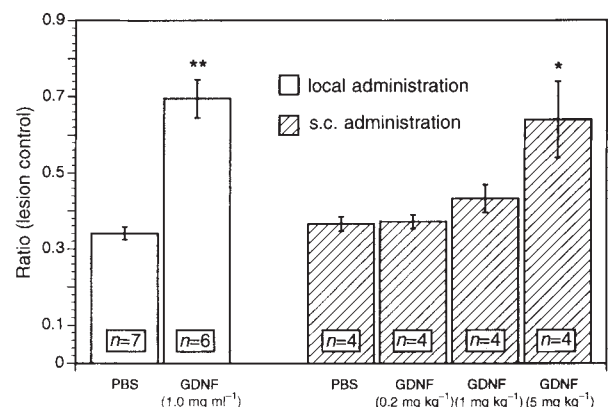


FIG. 3 GDNF prevents axotomy-induced decrease of AChT in adult motor neurons. AChT immunoreactivity of the facial nucleus after local and subcutaneous (s.c.) administration of PBS or GDNF for 7 days is shown. Values are means $\pm$ s.e.m. GDNF significantly attenuated axotomy-induced decrease of AChT immunoreactivity in the lesioned facial nucleus by local administration (** $P < 0.0001$, 2-tailed Student *t*-test) or by subcutaneous administration (* $P < 0.01$ for 5 mg kg⁻¹ of GDNF versus PBS; ANOVA followed by Dunnett *t*-test).

METHODS. The right facial nerves of adult female rats were transected near the stylo-mastoid foramen. For local administration, a ($3 \times 3 \times 3$ mm³) piece of Gelfoam soaked in 1 mg ml⁻¹ GDNF in PBS ($n = 6$) or PBS alone ($n = 7$) was implanted into the lesion site at the time of surgery. For subcutaneous treatment, GDNF in PBS at the doses of 0, 0.2, 1 and 5 mg per kg body weight were injected daily behind the neck of rats ($n = 4$ for each dose). Seven days after the facial nerve transection, animals were killed and perfused transcardially with PBS followed by 4% paraformaldehyde in 0.1 M sodium phosphate buffer, pH 7.2. Brainstems containing the facial nucleus were cut into 60- μ m serial coronal sections and processed for immunohistochemistry with either mouse monoclonal antibody against AChT (ascites, 1:500) or a control mouse myeloma IgG, followed by 2 μ g ml⁻¹ of secondary biotinylated horse anti-mouse and the ABC method²¹. The relative intensity of AChT immunoreactivity was determined by obtaining the mean grey-scale intensity for each outlined nucleus minus the background staining of adjacent AChT negative grey matter as described³. As local and subcutaneous GDNF treatments did not affect AChT immunostaining of the non-lesioned facial nucleus, data are expressed as the ratio of the relative optical density of lesioned over non-lesioned facial nuclei of the same sections.

suggests that GDNF selectively acts on certain neuronal populations in the peripheral nervous system.

A variety of neurotrophic factors, including ciliary neurotrophic factor (CNTF), BDNF, NT-3 and NT-4/5 have been shown to influence motor neuron survival *in vivo*. In the neonatal rat facial nerve axotomy model, motor neurons surviving after lesion increased from 19 to 76% with CNTF⁵, from 13 to 47%¹⁰ or 28 to 82% with BDNF¹¹, 13 to 28% with NT-3 (ref. 10) and 28 to 70% with NT-4/5 (ref. 11). As shown here, GDNF increased motor neuron survival after axotomy from 6% to almost 100%. In addition, GDNF completely prevents lesion-induced atrophy in neonatal motor neurons. This has not been observed before with members of the neurotrophin family^{10,19}. In the adult rat, GDNF has a potency similar to BDNF³ in preventing the axotomy-induced decrease of AChT immunoreactivity in the facial nucleus. As GDNF, members of the neurotrophin family and CNTF belong to different molecular families and act on different receptors, their potential additive or synergistic effects on motor neuron survival and function should be evaluated in animal models.

In conclusion, we have shown that GDNF, a member of the transforming growth factor- β superfamily, has survival-promoting effects on motor neurons *in vivo*. This finding, together with those in a related report¹⁸, suggests that GDNF is important

for controlling motor neuron survival during development and maintenance of motor neuron function in the adult. □

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Developing motor neurons rescued from programmed and axotomy-induced cell death by GDNF

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DURING normal development of the vertebrate nervous system, large numbers of neurons in the central and peripheral nervous system undergo naturally occurring cell death¹. For example, about half of all spinal motor neurons die over a period of a few days in developing avian, rat and mouse embryos¹. Previous studies have shown that extracts from muscle and brain, secreted factors from glia, as well as several growth factors and neurotrophic agents, including muscle-derived factors, can promote the survival of developing motor neurons *in vitro* and *in vivo*^{2–15}. But because neurotrophins and other known trophic agents administered alone or in combination are insufficient to rescue all developing motor neurons from cell death⁸, other neurotrophic molecules are probably essential for the survival and differentiation of motor neurons. Here we report that glial-cell-line-derived neurotrophic factor (GDNF), a potent neurotrophic factor that enhances survival of mammalian midbrain dopaminergic neurons^{16,17}, rescues developing avian motor neurons from natural programmed cell death *in vivo* and promotes the survival of enriched populations of cultured motor neurons. Furthermore, treatment with this agent *in vivo* also prevents the induced death and atrophy of both avian and mouse spinal motor neurons following peripheral axotomy.

Daily treatment of chick embryos *in ovo* with 5 and 10 μ g of GDNF from embryonic day (E)6 to E9 (the main period of

normal motor neuron death) rescued a significant number of motor neurons (25%) from programmed cell death (PCD) (Table 1). Lower doses were ineffective and chronic treatment with higher doses (20 μ g per day for example) was lethal. A small but significant number of motor neurons continued to undergo normal PCD between E9 and E12, and GDNF treatment (5 μ g daily, E9 to E15) was also effective during this later period (Table 1). Cell death of other peripheral neurons was also examined after daily GDNF treatment (5–10 μ g) from E6 to E9 or E9 to E15 (ciliary ganglia, CG). Although neuron numbers were significantly increased in sympathetic ganglia (SG), GDNF was ineffective in rescuing dorsal root, nodose or ciliary neurons following treatment during the principal period of cell death in these ganglia (Table 1). In apparent contrast to these *in vivo* results with avian embryos, GDNF promotes the survival of cultured rat embryonic nodose neurons but is without effect on rat SG neurons *in vitro*¹⁸. This disagreement could be due to the use of different species or to differences in *in vitro* and *in vivo* methods. Several populations of neurons in the brain of avian embryos undergo normal PCD between E9 and E16 (ref. 8) but, with the exception of the isthmo-optic nucleus (ION)¹⁹, specific neurotrophic factors have not yet been identified for any of these cell types. Of the ten nuclei examined here, only oculomotor (n.III) and trochlear motor neurons (n.IV), and interneurons in the ION, showed a small, but statistically significant increase in the number of cells after GDNF treatment (Table 1). Tyrosine hydroxylase (TH) immunocytochemistry was used to examine the effect of GDNF on neuron numbers and differentiation in the avian substantia nigra (TPc: nucleus tegmenti pedunculo-pontius, pars compacta)²⁰. Although it is not known whether dopaminergic neurons in the TPc undergo normal PCD, GDNF treatment from E9 to E15 failed to increase the number of TH-positive neurons in the TPc on E16 (Table 1). GDNF also had no apparent effect on cell size or on branching of neuronal processes of TH-positive TPc neurons, or on the intensity of TH immunolabelling (data not shown).

Axotomy of avian spinal motor neurons on E12 following the cessation of normal PCD results in an additional motor neuron loss of 50% by E16, as well as the atrophy of surviving motor neurons. Treatment with GDNF after axotomy completely prevented both cell loss and atrophy (Table 2). Similarly, axotomy

TABLE 1 Neuron numbers (mean $\pm$ s.d.) on E10 and E16 following treatment of avian embryos with GDNF

	E10				E16	
	Control	0.5 μ g	5 μ g	10 μ g	Control	5–10 μ g
MN	12,227 $\pm$ 976 (8)	11,975 $\pm$ 1,225 (5)	16,218 $\pm$ 885* (8)	16,510 $\pm$ 1,154* (6)	11,567 $\pm$ 1,043 (5)	13,019 $\pm$ 876† (5)
DRG	9,876 $\pm$ 610 (8)	10,234 $\pm$ 750 (5)	10,516 $\pm$ 821 (8)	9,917 $\pm$ 1,011 (6)	8,521 $\pm$ 1,133 (5)	9,016 $\pm$ 959 (5)
SG	7,529 $\pm$ 743 (8)	7,290 $\pm$ 817 (5)	9,511 $\pm$ 856† (8)	9,433 $\pm$ 614† (6)	6,157 $\pm$ 638 (5)	7,949 $\pm$ 820‡ (5)
CG	—	—	—	—	2,897 $\pm$ 320 (7)	3,019 $\pm$ 425 (6)
NG	5,449 $\pm$ 351 (5)	—	—	5,323 $\pm$ 540 (5)	—	—
n.III	—	—	—	—	2,200 $\pm$ 219 (8)	2,719 $\pm$ 271† (5)
n.IV	—	—	—	—	621 $\pm$ 73 (8)	859 $\pm$ 61* (8)
n.V	—	—	—	—	1,315 $\pm$ 106 (8)	1,450 $\pm$ 147 (8)
m.V	—	—	—	—	1,207 $\pm$ 270 (8)	1,139 $\pm$ 188 (6)
n.VI	—	—	—	—	856 $\pm$ 86 (8)	917 $\pm$ 90 (5)
n.VII	—	—	—	—	427 $\pm$ 51 (8)	486 $\pm$ 42 (5)
AON	—	—	—	—	977 $\pm$ 74 (8)	1,113 $\pm$ 81 (6)
ION	—	—	—	—	8,141 $\pm$ 810 (8)	10,195 $\pm$ 716‡ (8)
LAM	—	—	—	—	1,177 $\pm$ 279 (8)	1,220 $\pm$ 306 (5)
MAG	—	—	—	—	3,814 $\pm$ 410 (8)	4,122 $\pm$ 320 (5)
TPc	—	—	—	—	475 $\pm$ 77 (4)	510 $\pm$ 81 (4)

Embryos were treated daily with human recombinant GDNF¹⁶ by administration onto the chorio-allantoic membrane through a window in the shell as described⁸. Although human recombinant GDNF used for these experiments is non-glycosylated (M_r 30K), the bioactivity of native and recombinant GDNF is indistinguishable in culture (L.-F. Lin, personal communication). Various doses of GDNF dissolved in 50 μ l avian Tyrodes solution were administered from E6 to E9 or from E9 to E15 and embryos were killed on either E10 or E16. The lumbar spinal cord with attached DRG and SG, and the NG, CG and entire brain were dissected, fixed in either Bouin's solution, and processed for paraffin histology as described⁸. All tissues were sectioned at 8–10 μ m and stained with thionin. Neurons were counted blind in every fifth or tenth section as described⁸. In the DRG and SG, neurons were counted in only one ganglion (L3). All other neurons were counted in serial sections through each entire nucleus or ganglion. TH immunocytochemistry was performed using a rabbit antibody against TH (TE-101, Eugene Tech) following perfusion fixation with 4% paraformaldehyde. Serial transverse cryostat sections (15 μ m) were made through the entire brain and stained as described¹⁶. All TH-positive neurons within the boundaries of the avian substantia nigra (TPc), as described by adjacent Nissl stained sections, were counted; * $P < 0.002$; † $P < 0.003$; ‡ $P < 0.02$ (t-tests). Abbreviations: MN, motor neurons; DRG, dorsal root ganglion; CG, ciliary ganglion; CG, sympathetic ganglion; NG, nodose ganglion; n.III, oculomotor; n.IV, trochlear; n.V, trigeminal motor; m.V, mesencephalic sensory nucleus of the trigeminal; n.VI, abducens; n.VII, facial motor; AON, accessory oculomotor nucleus; ION, isthmo-optic nucleus; LAM, nucleus laminaris; MAG, nucleus magnocellularis; TPc, nucleus tegmenti pedunculo-pontius, pars compacta.

of mouse spinal motor neurons on postnatal day (P)5 (normal PCD occurs between E14 and E18; refs 21, 22) results in a 40–50% induced cell loss and significant atrophy by P12 (ref. 23); GDNF treatment also completely prevented both axotomy-induced cell death and motor neuron atrophy (Table 2). It is interesting that, in the mouse, GDNF not only prevents axotomy-induced atrophy, but also results in hypertrophy of the lesioned motor neurons (Table 2). Because GDNF did not have this effect on non-lesioned motor neurons, injured mouse motor neurons apparently have an increased responsiveness to GDNF. In the chick and mouse, GDNF is as effective as other neurotrophic factors (for example, IGF-1, BDNF, CNTF) in rescuing motor neurons from axotomy and in preventing atrophy²³.

To examine whether the effect of GDNF on the normal PCD of avian motor neurons *in vivo* was direct or mediated by other cell types, spinal motor neurons were isolated and cultured *in vitro*. GDNF had a striking dose-dependent effect on the survival of cultured motor neurons (Fig. 1). Doses as low as 1 ng ml⁻¹ were effective, with 10 ng ml⁻¹ being saturating. About 63% of the motor neurons present after plating (Fig. 1) were maintained for 2 days by 10 ng ml⁻¹ GDNF, compared to less than 30% in control cultures. A previously identified motor neuron trophic

factor, ciliary neurotrophic factor (CNTF)^{4,8} was 2–3-fold less potent than GDNF in promoting survival in this *in vitro* assay (Fig. 1). By contrast, optimal doses of embryonic avian muscle extract^{7,8} were generally more effective than GDNF (data not shown). Because our motor neuron cultures were enriched but not pure (Fig. 1), we cannot exclude an indirect effect of GDNF; however, GDNF has comparable survival-promoting effects on purified populations of cultured rat¹⁸ and avian motor neurons (C. Henderson, personal communication). GDNF thus appears to be more potent than either the neurotrophins or CNTF in directly promoting the survival of cultured motor neurons⁵. By contrast, GDNF is only slightly more potent than the neurotrophins, and is equivalent to CNTF and muscle extract, in preventing the PCD of avian motor neurons *in vivo*^{8,24}.

GDNF is a glycosylated homodimer of relative molecular mass ~33–45K and is a member of the transforming growth factor- β superfamily¹⁶. GDNF was purified and cloned from conditioned medium derived from a glial cell line, rat B49, on the basis of its survival and differentiation effects on cultured midbrain dopaminergic neurons¹⁶. Although the expression of GDNF messenger RNA in rat striatum^{17,25} is consistent with its proposed role as a target-derived trophic factor for midbrain

TABLE 2 Motor neuron numbers and soma size (mean $\pm$ s.d.) following axotomy in avian embryos and postnatal mice

	Chick			Mouse		
	Control (non-lesioned)	Saline	GDNF	Control (non-lesioned)	Saline	GDNF
No. of MN	3,210 $\pm$ 312 (19)	1,593 $\pm$ 175* (8)	2,776 $\pm$ 586§ (5)	800 $\pm$ 72 (4)	515 $\pm$ 56† (4)	815 $\pm$ 31‡ (4)
MN size (μm^2)	473 $\pm$ 94 (140)	399 $\pm$ 108† (70)	447 $\pm$ 83§ (70)	513 $\pm$ 78 (136)	434 $\pm$ 90† (42)	570 $\pm$ 92† (76)

Axotomy in chick embryos was performed on E12 by unilateral limb amputation of anaesthetized embryos as described²⁹. GDNF (5 μg) was administered daily from E12 to E15 onto the highly vascularized chorioallantoic membrane as described²⁹. Following fixation in Bouin's solution, the thoraco-lumbar spinal cord was removed, processed for paraffin histology, sectioned serially (12 μm) and stained with thionin or haematoxylin and eosin. All motor neurons in every tenth section through the L4 lumbar segment were counted blind as described²³. Motor neuron soma size was measured for cells in the middle of L4 that met all of the same criteria described for cell counts²³. The perimeter of motor neurons was drawn and digitized with a summagraphics bit pad interfaced to a microcomputer²³. Axotomy in postnatal mice was performed by removal of a length of sciatic nerve in anaesthetized animals on P5 as described²³. A small piece of gelfoam presoaked in 5 μg GDNF was apposed to the proximal nerve stump before skin suture. In addition, a direct injection of 5 μg GDNF was made into the lesion site on P9, and animals were killed and processed for histology on P12 as for chick embryos. All motor neurons in the fourth lumbar segment (L4) were counted in every fifth section²³. Sample sizes (animals or motor neurons) are shown in parenthesis. 'Control (non-lesioned)' refers to the side contralateral to the lesion. Animals receiving either saline or GDNF were examined separately for changes in motor neuron numbers (and size) on the contralateral side; because no differences were found, data from the contralateral side have been pooled. * $P < 0.009$ versus control; † $P < 0.001$ versus control; ‡ $P < 0.001$ versus saline; § $P < 0.001$ versus saline; || $P < 0.01$ versus control (t-test with Bonferroni correction). There were no significant differences in MN numbers in either chick or mouse between GDNF versus control, but in mouse, GDNF treatment resulted in the hypertrophy (increased MN size) of ipsilateral lesioned motor neurons compared to the contralateral (control) side.

dopaminergic neurons, the presence of GDNF mRNA in spinal cord^{17,26} and muscle¹⁸, and the receptor-mediated retrograde transport of GDNF from peripheral muscle to facial motor neurons in neonatal rats²⁷ indicates that it may also act on non-dopaminergic neurons. Our results, together with others on the trophic effects of GDNF on neonatal^{18,27} and adult rat facial motor neurons²⁷, are in fact consistent with a role for GDNF as a neurotrophic factor for motor neurons. Also, our results indicate that GDNF may act on other populations of non-dopaminergic avian neurons, including sympathetic neurons, cranial motor neurons and interneurons in the ION. Surprisingly, we were unable to detect any *in vivo* effects of GDNF on TH-

positive immunolabelled dopaminergic neurons in the developing avian substantia nigra.

In addition to their importance for understanding normal developmental events, neurotrophic factors that act on motor neurons are of potential use as therapeutic agents for motor neuron diseases and other pathologies involving motor neurons. So in addition to its possible application in the treatment of movement disorders involving dopaminergic neurons (Parkinson's disease for example)¹⁷, GDNF, either alone or in combination with other trophic factors²⁸, may also be useful in the treatment of motor neuron disorders like amyotrophic lateral sclerosis and spinal muscular atrophy. □

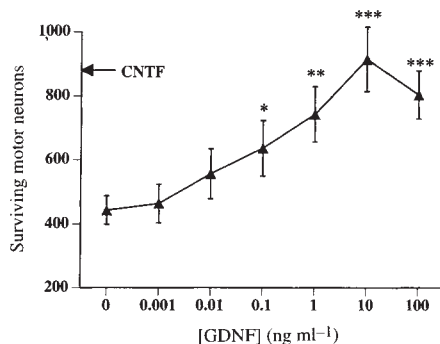


FIG. 1 The number of motor neurons (mean $\pm$ s.e.m.) per well after treatment with GDNF or a saturating (optimal) dose of CNTF (25 ng ml⁻¹). Spinal cord cells from the ventral one-half of the lumbar region of avian embryos were dissected on E5.5, dissociated and centrifuged over a metrizamide cushion^{2,30}, giving a total of $\sim 30,000$ motor neurons per lumbar cord. Enrichment for motor neurons afforded by this procedure varied somewhat between experiments, but on average was 70–80%, based on immunolabelling with a motor-neuron-specific marker, islet-1 (ref. 18). Of the $\sim 2,000$ cells initially plated per well, 90% remained viable 3–6 h after plating, 70–80% of these being motor neurons. Two days after plating, motor neurons were counted in ten predetermined 20 $\times$ objective fields and the total number of motor neurons per well was estimated from these samples³⁰. Only apparent healthy, viable cells with neurites were included in the counts. Each dose of GDNF (and CNTF) was tested in 10–12 separate replications. Values represent the average of these several replicate experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus control (0) (t-tests with Bonferroni correction).

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***W*/*kit* gene required for interstitial cells of Cajal and for intestinal pacemaker activity**

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THE pacemaker activity in the mammalian gut is responsible for generating anally propagating phasic contractions. The cellular basis for this intrinsic activity is unknown. The smooth muscle cells of the external muscle layers and the innervated cellular network of interstitial cells of Cajal, which is closely associated with the external muscle layers of the mammalian gut, have both been proposed to stimulate pacemaker activity^{1–5}. The interstitial cells of Cajal were identified in the last century but their developmental origin and function have remained unclear. Here we show that the interstitial cells of Cajal express the Kit receptor tyrosine kinase. Furthermore, mice with mutations in the dominant *white spotting* (*W*) locus, which have cellular defects in haematopoiesis, melanogenesis and gametogenesis⁶ as a result of mutations in the *Kit* gene^{7,8}, also lack the network of interstitial cells of Cajal associated with Auerbach's nerve plexus and intestinal pacemaker activity.

Injection of antibodies directed against the extracellular domain of the Kit receptor tyrosine kinase into newborn mice leads to changes in *in vitro* contraction patterns in the small intestine and absence of Kit messenger RNA in the myenteric plexus area⁹. This observation prompted us to investigate whether the Kit receptor might play a role as a signalling molecule required for the development of the interstitial cells of Cajal (ICC) and therefore be essential for intestinal pacemaker activity. To determine whether mutations at the murine *W*/*kit* locus might affect normal development of ICC, we first examined the morphology of the intestines of mutant *W*/*W*^v mice and their control littermates. Control mice (albino, *+/+* and *W*/*+*) had normal ICC networks in the small intestine, visualized by selective uptake of methylene blue (Fig. 1a) and confirmed by electron microscopy (Fig. 2a, b). By contrast, the network of ICC in the myenteric plexus region was absent in *W*/*W*^v mice (Fig. 1b), again confirmed by electron microscopy (Fig. 2c, d). In the mutants, only scattered methylene-blue-positive cells which might be genuine ICC were observed (Fig. 1b). Using electron microscopy, we counted 51 ICC along a length of 1,109 cross-sectioned muscle cells bordering the myenteric plexus in *W*/*+* control mice; by contrast, only 7 ICC were observed along a length of 3,059 muscle cells in *W*/*W*^v mice. Quantitatively, 70% of muscle cells bordering the Auerbach's plexus contacted ICC in control mice, compared with only 1.5% in *W*/*W*^v mice. We also observed the direct apposition of large stretches of the circular and longitudinal smooth muscle layers in *W*/*W*^v mice in the absence of the intermediary network of ICC found in normal animals (Fig. 2c). Light and electron microscopic analysis of duodenal muscularis revealed no differences in the density, morphology or ultrastructural features of enteric neurons or glial cells between *+/+* and *W* mutant mice, indicating

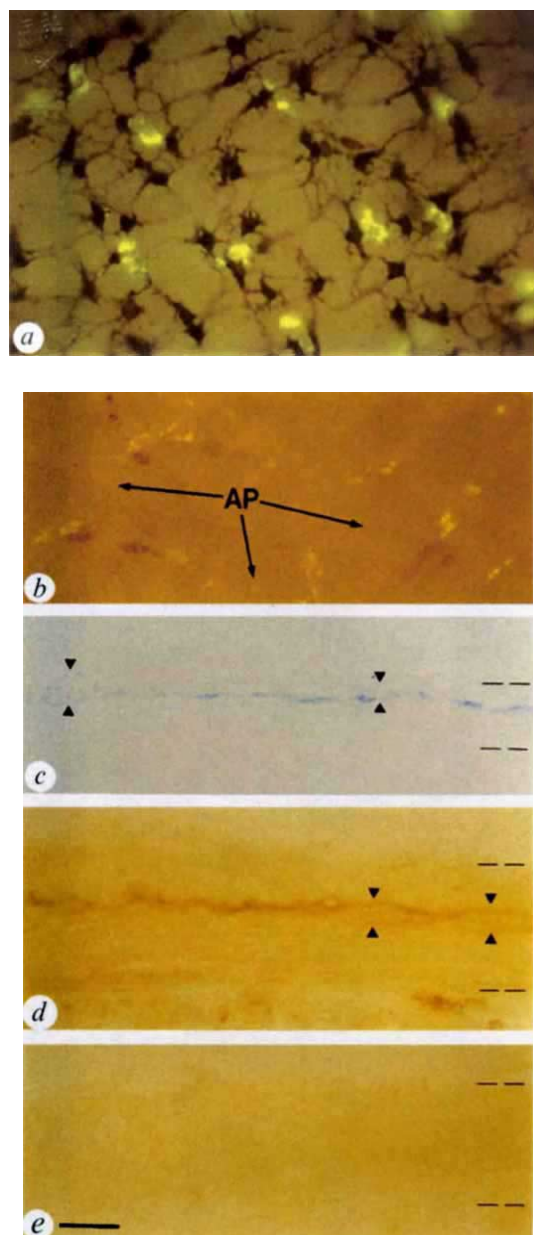


FIG. 1 Histochemical analysis of the small intestine in wild-type and *W* mutant mice. a, b, Double staining of interstitial cells of Cajal associated with Auerbach's plexus (ICC) (shown as the stained cellular network using vital staining with methylene blue) and macrophages (fluorescence; ingestion of fluorescein isothiocyanate FITC-labelled dextran)¹⁹. Whole mounts of the isolated duodenal muscularis externa of a control albino mouse (a) and *W*/*W*^v mouse (b); a is representative of the normal staining pattern of both cell types in albino mice as well as *W*/*+* mice. Normally, ICC together with macrophages form a partial sheath around ganglia and primary fascicles of Auerbach's plexus (AP) but in b, only macrophages are present in normal numbers and normally organized. c–e, Sectioned jejunal tissue. c, 1 μ m section of Epon-embedded tissue, processed with preservation of methylene blue, no post-staining. ICC are seen as the blue, broken line between the two layers of the muscularis externa (between dashed lines). Arrowheads point to unstained, partially enveloped elements of Auerbach's plexus (compare with a). d, e, Frozen 8- μ m sections, showing Kit immunoreactivity in ICC in a *W*/*+* mouse and its absence in the *W*/*W*^v mouse (e). Magnification, $\times 225$ (a); $\times 320$ (b–e); scale bar, 25 μ m. METHODS. Methylene blue was preserved through dehydration and embedding by precipitation as a hexachloroplatinate²⁰. Immunocytochemistry was performed on unfixed frozen sections of small intestines from *W*/*W*^v mice and their controls. Both the PAP and the biotin/streptavidin/Texas-red methods were used²¹.

that defects in the Kit signalling pathway do not affect neuronal or glial cells in the Auerbach's plexus region.

These data indicate that a functional Kit receptor is required for the development of ICC in the Auerbach's plexus region of the small intestine. To determine whether the ICC deficiency in *W* mutant mice was a cell-autonomous defect, we analysed Kit RNA and protein expression in *W* mutant mice and control animals. We used a polyclonal antibody directed against the intracellular domain of the Kit receptor tyrosine kinase to localize the protein. In wild type and *W^x/+*, high levels of Kit expression were observed between the longitudinal and the circular muscle layers at the level of Auerbach's plexus (Fig. 1d). The stained cells surrounded the ganglia and their distribution in the

Auerbach's plexus area was identical to that of the methylene-blue-stained ICC (Fig. 1c). By contrast, no Kit immunoreactivity was found in or between the muscle layers of *W^x/W^y* mice (Fig. 1e).

We next performed whole-mount RNA *in situ* experiments on the ileum of wild-type and *W/W^y* mice. The ileum of *+/+* mice contained Kit-positive cells whose organization was identical to that of ICC, as revealed by methylene-blue staining (Fig. 3a). These cells were localized between the longitudinal and circular

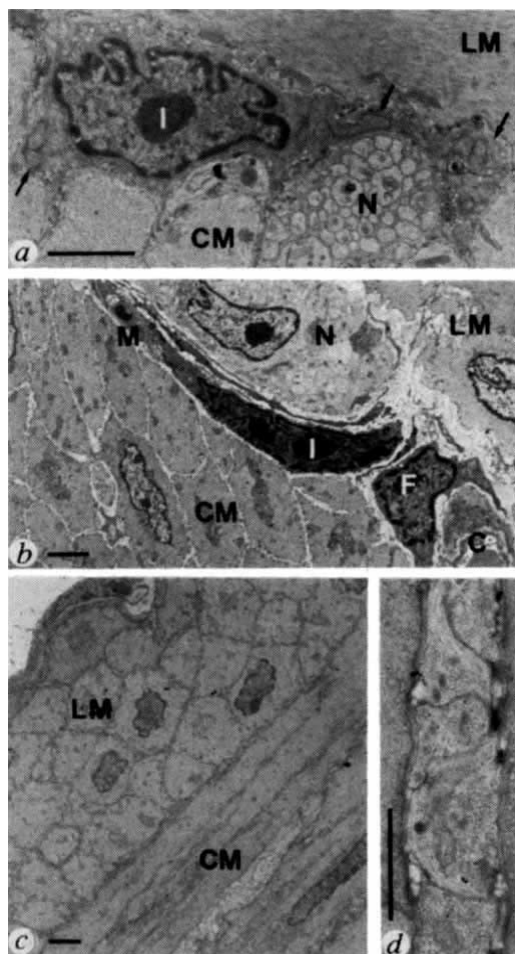


FIG. 2 EM analysis of the small intestine of wild-type and *W* mutant mice. *a–d*, Electron micrographs of the jejunal myenteric plexus area in an albino mouse (*a*), a *W^x/+* mouse (*b*), and a *W^x/W^y* mouse (*c, d*). *a*, Normal appearance of ICC in the Auerbach's plexus region (*I*), tightly associated with nerve fascicle (*N*) of Auerbach's plexus, between the longitudinal (*LM*) and circular (*CM*) muscle layers. An abundance of mitochondria (arrows) and caveolae are distinctive ultrastructural features of ICC. *b*, Prestaining with methylene blue assists the differentiation of ICC (*I*, with increased granularity and electron-density of nuclei and ribosomal areas) from fibroblasts (*F*), macrophages (*M*, macrophage process) and pericytes (*C*, capillary). *c–d*, The overall organization of the muscle layers seemed normal. Although cell types other than ICC were counted in normal numbers in *W/W^y*, one result of the absence of ICC was a strong increase in the extension of areas of direct apposition of muscle cells of LM and CM (*c*). Sheath cells around nerves were either absent (*d*, small tertiary nerve between the muscle layers) or had fibroblast ultrastructure.

Tissues were fixed and processed for electron microscopy (Philips 300 microscope) by routine methods⁵. Scale bars: 2 μ m (*a–c*); 1 μ m (*d*).

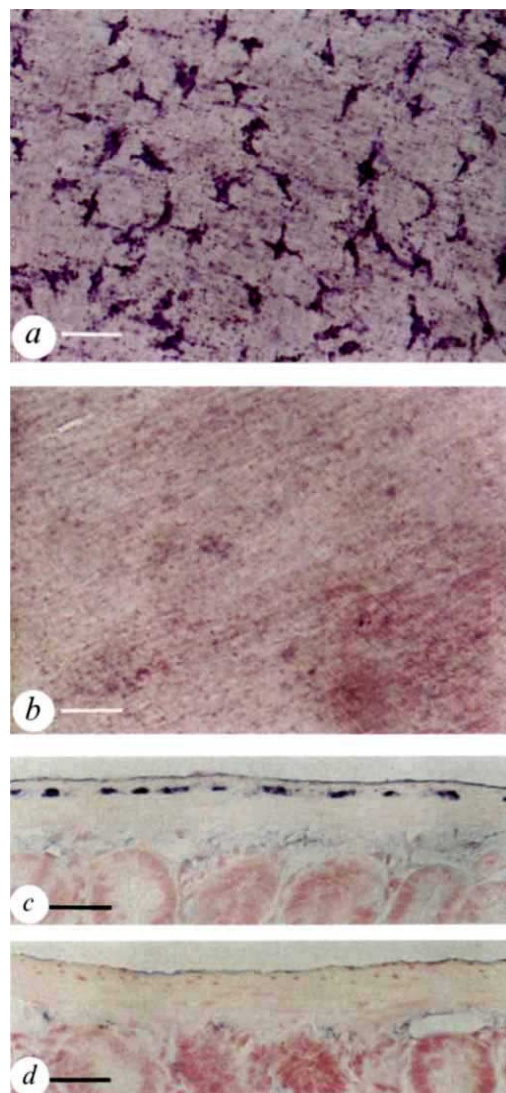
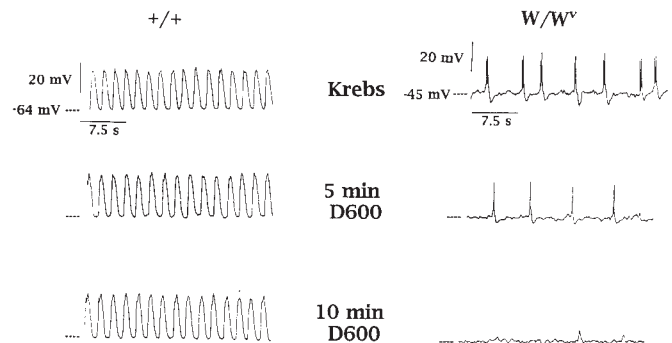


FIG. 3 Whole-mount RNA *in situ* analysis of *kit* expression in the small intestine of wild-type and *W* mutant mice. *a*, A network of Kit-expressing cells is present in the isolated external muscle layers in *+/+* mice, identical in appearance to the ICC network (compare Fig. 1a). *b*, Kit-positive cells were absent in similar whole mounts of *W/W^y* mice. Cross-sectioning of the whole-mount tissues localized the Kit-positive cells in *+/+* mice between the longitudinal and circular muscle layer (*c*; compare Fig. 1c at the same magnification) and confirmed their absence in *W/W^y* mice (*d*). Scale bars: 50 μ m (*a, b*); 25 μ m (*c, d*).

METHODS. Whole-mount RNA *in situ* hybridization was performed according to the method of D. Henrique and D. Ish-Horowitz (personal communication), with the following modifications. Proteinase K digestion was for 30 min at a concentration of 20 μ g ml⁻¹. The hybridization mix contained 5 $\times$ SSC, 50% formamide, 5 mM EDTA, 50 μ g ml⁻¹ yeast tRNA, 0.2% Tween-20, 0.5% CHAPS and 100 μ g ml⁻¹ heparin, pH 6.5 with citric acid. After *in situ* hybridization, tissues were postfixed in 4% paraformaldehyde before wax embedding and sectioning. The *kit* cDNA probe has been described¹¹.

FIG. 4 Action potential generation in wild-type and *W* mutant mice. The left panel depicts the slow-wave-type action potentials generated by $+/+$ mice. Slow-wave-type action potentials were generated at a constant frequency. L-type calcium channel blockers did not have any influence on the slow-wave component. Occasionally spikes were superimposed on the slow wave and these were abolished by L-type calcium channel blockers. The right panel depicts typical spike-like action potentials generated by the small intestinal smooth muscle cells of *W/W^v* mice. Fast spikes occurred as single spikes or in groups of 2–3. These spikes were completely abolished by the action of L-type calcium channel blockers.

METHODS. Female mice were killed by cervical dislocation. The small intestine was exposed by a midline abdominal incision and a 3–5-cm segment was removed, 1 cm from the gastroduodenal sphincter. The segment was placed in a dissecting dish filled with oxygenated (95% O_2 and 5% CO_2) Krebs solution and opened flat. It was then pinned to the Sylgard bottom of a dissecting dish and the mucosa removed by sharp dissection. Muscle strips, 15 mm long, were prepared by cutting them parallel to the longitudinal muscle bundles: the tissue was 1.5 mm wide over a length of 10 mm and gradually widened to 3 mm at the proximal end. At the wide end an area of the tissue (3 × 3 mm) was pinned to the Sylgard bottom of a transfer holder. The preparation was mounted by putting insect pins along all four edges. This transfer holder with the tissue attached was then placed in an organ bath at 36.0–37.0 °C. The end of the tissue that was not pinned was tied to a force transducer to record mechanical contraction. Intracellular recordings were made using microelectrodes with 30–60 M Ω tip resistance because microelectrodes with a tip resistance within this range have a tip diameter of ~150 nm, which was appropriate for impaling cells. Microelectrodes were prepared from 1.2 mm outside-diameter glass capillaries (WPI) and filled with 3 M KCl. A microelectrode was inserted into a microelectrode holder (WPI M700P) connected to an electrometer (WPI M-707A) which was a high-impedance probe.



muscle layers in an identical position to the ICC (Fig. 3c). By contrast, no Kit-positive cells were observed in the muscle layers of *W/W^v* mice (Fig. 3b, d).

To determine whether these morphological and cellular differences between $+/+$ and *W* mutant mice were associated with functional anomalies, we measured the electrical activity of the small intestinal muscle layers. Normal mice displayed slow-wave-type action potentials with an amplitude of 21.5 ± 5.9 mV, a frequency of 32.4 ± 1.0 cycles per minute (range 30–36 c.p.m.) and a resting membrane potential of -60.0 ± 3.0 mV (Fig. 4; $n=9$). By contrast, the ilea of *W/W^v* mice failed to display any slow-wave-type action potentials (Fig. 4). The membrane potential was -44.8 ± 1.3 mV ($n=14$) and at irregular frequency fast spike-like action potentials arose from it, singly or in groups of 2–6. Their amplitude was 16.4 ± 2.1 mV, the frequency ranged from 4 to 20 c.p.m., and was irregular within one preparation. In 4 out of 14 *W/W^v* mice, no spontaneous action potentials were observed. The slow-wave component or pacemaker activity of gut smooth muscle is insensitive to L-type calcium channel blockers¹⁰. In the presence of the blockers nifedipine or D600, the slow-wave component of the action potentials in $+/+$ mice remained unaltered, whereas the electrical activity of *W/W^v* mice was completely abolished ($n=14$; Fig. 4).

These data demonstrate that mutations at the murine *W* locus lead to the absence of the ICC network in the Auerbach's plexus region and of pacemaker activity in the small intestine, demonstrating an essential role for ICC in gut pacemaker activity. Because the ICC express the Kit receptor, we conclude that the absence of these cells in *W* mutant mice reflects a direct role for Kit in ICC development. The phenotype of *W* mutant mice was thought to be restricted to cells of the haematopoietic, germ and melanocyte lineages⁶, even though Kit and its ligand Steel factor are contiguously expressed in additional anatomical sites, including the small intestine^{11,12}. Thus, our experiments extend the range of cell types affected by *W* mutations to include the interstitial cells of Cajal. Mice with mutations in Steel factor (*Sl/Sl^d* mutant mice) display abnormalities in the gut similar to those found here in *W* mutant mice (data not shown).

Our data suggest that the functional gut abnormalities and megacolon observed in individuals with piebaldism¹³, a hypopigmentation disorder that also results from mutation in the *kit* proto-oncogene^{14,15} reflect an identical function of the Kit signalling pathway in the development of the ICC in humans. Mutations in the *RET* proto-oncogene are also associated with gut abnormalities in humans (Hirschsprung's disease) and in gene-targeted mutant mice, as the result of a cellular deficit in neural-crest-derived enteric neurons^{16–18}. Thus, two members of the receptor tyrosine kinase family, *kit* expressed in ICC and *RET* expressed in neural-crest-derived ganglion cells, are both essential for normal gut function in mammals. □

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Developmental control point in induction of thymic cortex regulated by a subpopulation of prothymocytes

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T LYMPHOCYTES of the α/β T-cell receptor (TCR) lineage mature in the thymus, where they undergo a series of differentiation, expansion and selection events^{1–7}. For normal T-cell ontogeny to occur, thymocytes must interact physically with cortical and medullary thymic stroma cells^{8–10}. In parallel, interactions of the thymic stromal cells with TCR-positive thymocytes are necessary for the development of the thymic medulla^{10–12}. Comparable requirements for the differentiation of the cortex have not been defined, however. Here we analyse mutant mouse strains to assess the function of early prothymocytes in the induction of the thymic cortex. We find that animals with a developmental block at the earliest stage of T-lineage commitment lack a functional thymic cortex. This abnormality could be corrected in fetal but not adult animals by transplantation of either fetal or adult wild-type haematopoietic stem cells. Thus a developmentally restricted interaction of fetal stromal cells with early prothymocytes is required for the induction of a cortical microenvironment. In addition, a normal thymic architecture is necessary for sustained T-cell ontogeny.

Mice homozygous for the human CD3- ϵ transgene, tg ϵ 26 (ref. 13), were compared with RAG-2^{-/-} (null) mutant mice¹⁴ to analyse the structure and function of the thymic microenvironment. Transgenic (tg) ϵ 26 animals have an almost complete arrest very early in T-cell development; only 2–3% of thymocytes progress to the CD44⁺CD25⁻ phenotype^{13,15,27}. By contrast, thymocytes from RAG-2^{-/-} mice are arrested later in T-cell development, at the stage of CD44⁺CD25⁺ expression^{14,16,17}. The minute thymuses (~1% of wild-type size) from ϵ 26 mice were compared to RAG-2^{-/-} thymuses by immunohistology. Using monoclonal antibodies specific for cortical (ER-TR4) and medullary (ER-TR5) thymic epithelial cells, we found that cortical epithelial cells were arranged parallel to the thymic capsule, in contrast to the typical radial grouping in normal thymuses (Fig. 1A, a) and that medullary epithelial cells were found in clusters randomly distributed throughout the thymic rudiment (Fig. 1A, a)^{18,19}. Dendritic cells and other thymic stromal cells were also identified in the tg ϵ 26 thymus (data not shown). We also noticed large macrophage-containing cysts (Fig. 1A), a marked increase in the number of fibroblasts, focal accumulations of mature B lymphocytes (B220⁺ IgM⁺) and an increased expression of major histocompatibility complex (MHC) class I and II molecules on epithelial cells (Fig. 1A; W.v.E. *et al.*, manuscript in preparation). The abnormal thymic architecture could not have been caused by inappropriate expression of the transgene in thymic stromal cells or early thymocytes because neither cell type expressed the human CD3- ϵ gene, as assessed by immunohistology (Fig. 1B). Thus, the structural defect of the thymic microenvironment might be due to a lack of T-cell maturation. Compared to ϵ 26 mice, analysis of RAG-

2^{-/-} mice revealed a densely packed and normally organized cortex as well as a small but distinct medulla (Fig. 1A, i, j). This striking difference in the microenvironment of tg ϵ 26 and RAG-2^{-/-} thymuses correlates with blocks at different control points of T-cell ontogeny^{13,14,16}.

To investigate whether the virtual absence of T-lineage-committed CD44⁺CD25⁻ thymocytes accounts for the failure to develop a normal cortex and medulla, we transferred T-lymphocyte-depleted bone marrow cells from wild-type (C57BL/6 $\times$ CBA/J)F₁ animals into adult tg ϵ 26 and RAG-2^{-/-} mice. Four weeks after transplantation, T cells of donor origin (H-2^{b $\times$ k}) could be detected in the peripheral blood of tg ϵ 26 mice (H-2^k) (Fig. 2a). However, lymph nodes and spleen showed a reduction of up to 80% in the relative frequency of T lymphocytes (Table 1). The majority of peripheral T cells were CD4⁺ and displayed a phenotype typical of activated cells (CD44⁺, CD69⁺ and CD62L⁻) (Table 1)^{20,22}. When tested six weeks after transplantation, lymphocytes from tg ϵ 26 mice were severely deficient in their response to allogeneic splenocytes (Fig. 2b, c), although stimulation with concanavalin A resulted in proliferation levels comparable to those from normal mice (data not shown). By contrast, the peripheral lymphoid compartment of transplanted RAG-2^{-/-} mice was completely repopulated by functional T cells (Table 1 and Fig. 2b, c). Thus, bone-marrow transplantation did not reconstitute a functional T-cell compartment in adult tg ϵ 26 mice.

The thymuses of tg ϵ 26 mice that had received transplants contained only slightly more cells than those of tg ϵ 26 control animals (2–4 $\times$ 10⁶ compared to 1–2 $\times$ 10⁶ cells). Immunohistology of the thymic microenvironment of reconstituted tg ϵ 26 mice revealed only a partial regrouping of cortical and medullary epithelial cells and a continuous lack of discrete areas (Fig. 1A, c and d). Furthermore, both the B-cell foci lined by ER-TR5⁺ cells and the cysts persisted. By contrast, RAG-2^{-/-} mice that had received transplants displayed a normally structured thymic architecture with an average of 8 $\times$ 10⁷ thymocytes (Fig. 1A, k and l).

Phenotypic analysis by flow cytometry of thymocytes from transplanted tg ϵ 26 mice revealed a decrease of CD4⁺CD8⁺ thymocytes and a relative increase of single-positive T cells (Fig. 3a, b). These CD4⁺ and CD8⁺ thymocytes also had an increased surface expression of the TCR/CD3 complex, the CD69 and MHC class I antigens (data not shown) and therefore represented mature cells. Over time, CD4⁺CD8⁺ thymocytes diminished in relative and absolute numbers in transplanted tg ϵ 26 animals whereas the relative percentage of phenotypically mature CD4⁺ and CD8⁺ cells increased. By contrast, the distribution of the four thymocyte subpopulations defined by CD4 and CD8 expression was normal in bone-marrow-transplanted RAG-2^{-/-} mice (Fig. 3e). Thus, transplantation of F₁ bone marrow into adult tg ϵ 26 mice could not restore a normal thymic microenvironment but resulted in abnormal T-lymphocyte differentiation and function.

As differentiation of the thymus anlage into a cortical and a medullary region occurs after day 13 of gestation^{5,23,24}, we examined whether the tg ϵ 26 thymus could be restored to normal by transplantation during fetal development. Thymuses prepared from tg ϵ 26 fetuses at day 16 of gestation were first grafted under the kidney capsule of an adult tg ϵ 26 mouse and then transplanted with T-cell-depleted F₁ bone marrow cells. Up to 14 weeks later, the architecture of cortical and medullary domains in transplanted fetal thymus was normal (Fig. 1A, e and f) and there was a normal distribution of CD4⁺ and CD8⁺ thymocytes (Fig. 3c). By contrast, the orthotopic thymus in the same animal was abnormal in structure and size. Given this result, the possibility that undetectable T-cell progenitor cells expressing the transgene could actively interfere with normal development of the thymic cortex of unmanipulated tg ϵ 26 mice seems extremely unlikely because such cells would have to populate only the adult thymus but not the transplanted fetal thymus

in double transplanted mice. Most of the T lymphocytes found in peripheral lymphoid tissues of double-transplanted tg ϵ 26 mice did not display phenotypic markers of activation (Table 1). These results suggest that inductive signals provided by prothymocytes beyond the developmental block of tg ϵ 26 mice, but before the stage of CD44⁺CD25⁺ cells, are essential for thymic epithelial cells to form distinct cortical and medullary microenvironments. The precise phenotype of the cells responsible for the induction of the cortex and the signals involved are not known. But transplanting normal bone marrow cells into tg ϵ 26 mice

leads to the development of a functionally competent precursor cell necessary for the induction of the thymic cortex. Mediation by soluble cytokines and direct cell-cell interactions may both be important²⁵, but cannot be demonstrated until the phenotype and function of the earliest T-cell-committed precursor cell is delineated.

To assess further the impact of prothymocytes on the development of the thymic cortex, we transplanted wild-type haematopoietic progenitor cells derived from fetal liver into day-16 tg ϵ 26 fetuses *in utero*. Six to fourteen weeks later, the thymuses

FIG. 1 Immunohistology of thymic tissue from bone-marrow-transplanted mice. **A**, Immunohistological staining for cortical (ER-TR4) and medullary (ER-TR5) thymic epithelial cells. **a, b**, Untransplanted tg ϵ 26 mice; **c, d**, T-cell-depleted (C57BL/6 $\times$ CBA/J)F₁ bone-marrow-transplanted adult tg ϵ 26 mice; **e, f**, adult tg ϵ 26 mice transplanted with day 16 fetal tg ϵ 26 thymus plus T-cell-depleted (C57BL/6 $\times$ CBA/J)F₁ bone marrow cells; **g, h**, tg ϵ 26 mice transplanted *in utero* with fetal congenic C57BL/6Thy1algM^a haematopoietic precursor cells; **i, j**, RAG-2^{-/-} mice; **k, l**, T-cell-depleted (C57BL/6 $\times$ CBA/J)F₁ bone-marrow-transplanted adult RAG-2^{-/-} mice. Tissue sections were stained for cortical (ER-TR4⁺; **a, c, e, g, i, k**) and medullary epithelial cells (ER-TR5⁺; **b, d, f, h, j, l**). The letters **b, m** and **c** refer to B cells, macrophages and the thymic capsule, respectively; the asterisk marks thymic cysts. The presence of B cells has been verified by immunohistology using mAb B220 on a sequential tissue section. The arrows in **j** point to ER-TR5⁺ epithelial cells. **B**, Immunohistology of thymic tissue from homozygous tg ϵ 26 mice (**a**) and heterozygous tg ϵ 600 mice (**b**) using anti-human CD3 ϵ mAb. White arrow indicates the thymic capsule.

METHODS. Tg ϵ 26 mice ($n=30-35$) and tg ϵ 600 mice ($n=3-5$) were generated by transgenic overexpression of DNA encoding the entire human CD3 ϵ gene, as described previously¹³. Heterozygous tg ϵ 600 mice display a normal thymic architecture and normal T-cell development, although their thymuses have ~50% fewer cells than those of non-transgenic litter mates. Homozygous recipient tg ϵ 26 (H-2^k) or RAG-2^{-/-} mice (H-2^b; kindly provided by F. Alt and M. Huang), age 8-12 weeks, were either pretreated with 5-fluorouracil (5-FU; 150 mg/kg⁻¹) 48 h before transplantation of 10×10^6 T-cell-depleted bone marrow cells from adult (C57BL/6 $\times$ CBA/J)F₁ donors (H-2^{kxb}) or received supralethal (1,100 rads) irradiation 1-2 h before bone marrow transplantation. Both treatments lead to identical results. Bone-marrow-transplanted tg ϵ 26 mice were analysed 4-9 weeks after transplantation. Unilateral transplantation of fetal day 16 (d16) thymic lobes under the kidney capsule of adult tg ϵ 26 mice was preceded 24 h earlier by injection of 5FU (150 mg/kg⁻¹) and followed one day later by injection of 10^7 T-cell-depleted (C57BL/6 $\times$ CBA/J)F₁ bone marrow cells. Thymus- and bone-marrow-transplanted mice were analysed 7-14 weeks after transplantation. For *in utero* transplantation, tg ϵ 26 fetuses were injected on day 15 of gestation. Haematopoietic progenitors (B220⁺, TER119⁻) from day-15 fetal liver were obtained by magnetic cell sorting (Miltenyi, Biotec) and injected through the uterine wall into the fetal liver (A.N. *et al.* manuscript in preparation). Transplanted mice were analysed 7-14 weeks after transplantation. For immunoperoxidase staining, thymic tissue was prepared as described^{18,19} and analysed with mAbs ER-TR4, ER-TR5, RA3-6B2, MOMA2, BM8, F4/80 and N418. The expression of human CD3 ϵ was assessed by immunofluorescence with FITC-labelled Mab (clone SK7). The background staining in **B, a** is nonspecific.

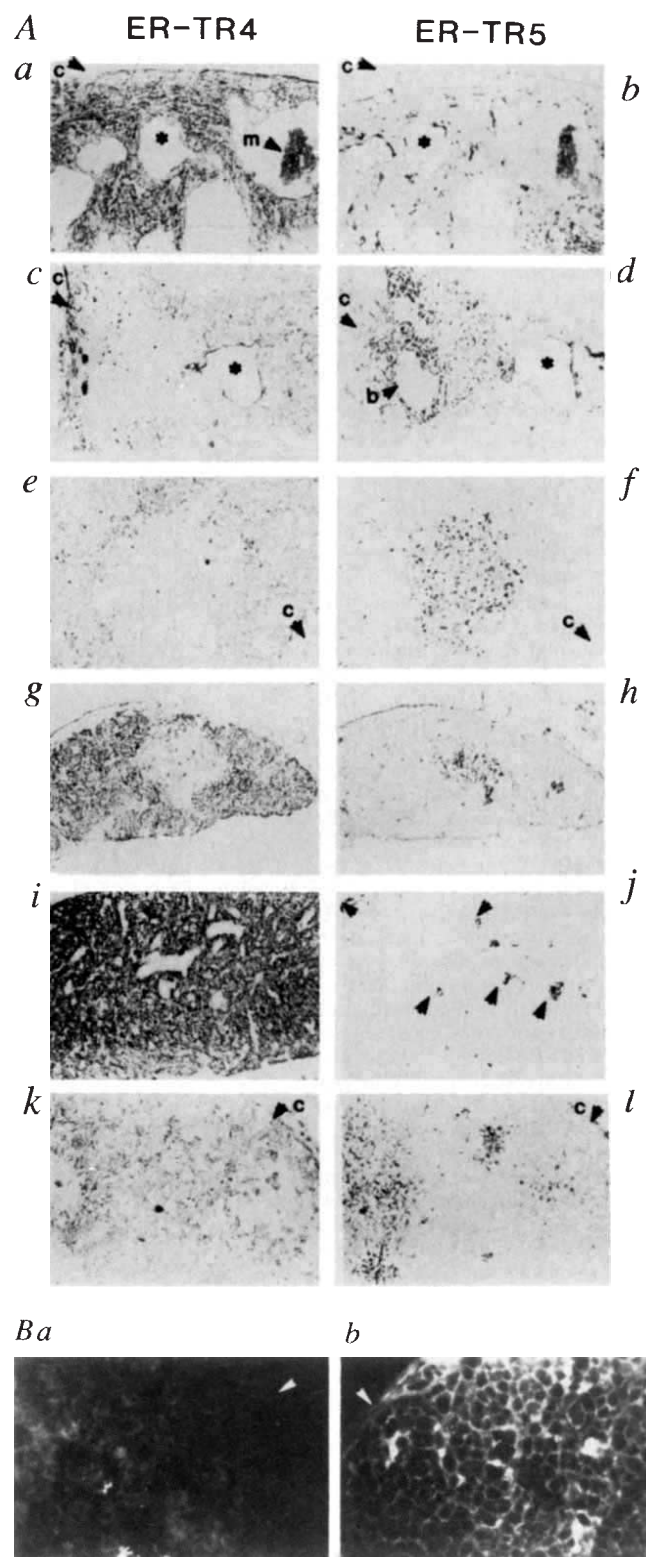


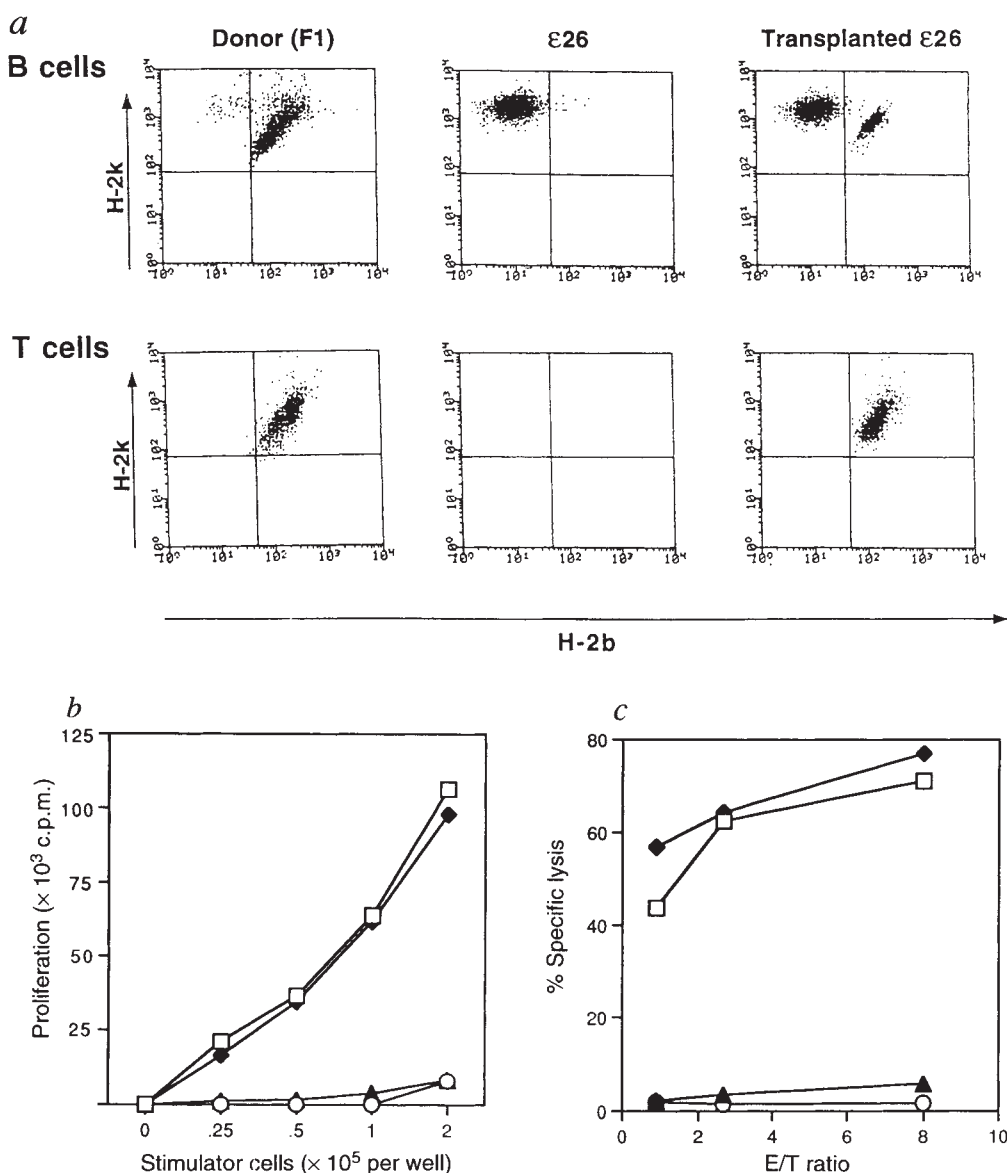
TABLE 1 Phenotypic analysis of peripheral T lymphocytes upon transplantation into transgenic $\epsilon 26$ mice

Transplantation		T-cell surface markers					
Recipient mice	Donor tissue		Percentage $\alpha\beta$ -TCR ⁺ cells*	Ratio of CD4 ⁺ /CD8 ⁺ cells	CD69 ⁺	Percentage of CD44 ⁺ TCR ⁺ cells	CD62L ⁺
tg $\epsilon 26$: adult (H-2 ^k)	(C57BL/6 $\times$ CBA/J)F ₁	1	35	7.8	23	75	20
	bone marrow	2	8	7.7	80	91	25
		3	13	4.7	64	68	33
	(C57BL/6 $\times$ CBA/J)F ₁	1	26	2.0	13	ND	77
	bone marrow and	2	34	3.8	44	31	67
		3	77	2.7	ND	50	80
tg $\epsilon 26$: d16 fetus (H-2 ^k)	tg $\epsilon 26$ fetal thymus						
	C57BL/6	1	82	2.3	16	15	60
	(H-2 ^b)	2	50	2.6	ND	ND	65
	fetal liver cells	3	38	1.0	ND	ND	85
RAG-2 ^{-/-} (H-2 ^b)	(C57BL/6 $\times$ CBA/J)F ₁	1	97	2.0	ND	ND	84
	bone marrow	2	75	2.4	23	12	90
		3	83	2.8	13	11	88
		3	83	2.8	13	11	88
(C57BL/6 $\times$ CBA/J)F ₁ (H-2 ^{b$\times$k})	none	1	100	1.8	10	19	91
		2	97	2.0	14	16	93
		3	87	2.4	9	10	92
		3	87	2.4	9	10	92

Mice were generated as described in Fig. 1 legend. Lymph node T cells were stained for the surface expression of α/β TCR (clone H57-597), CD4 (clone RM4-4), CD8 (clone 53-6.7), CD69 (clone H1.2F3), CD44 (Pgp-1; clone IM7) and CD62L (LECAM; clone MEL-14). ND, Not done.

* Values are given as percentage of wild-type.

FIG. 2 T cells in bone-marrow-transplanted transgenic $\epsilon 26$ mice. **a**, Peripheral T cells in bone-marrow-transplanted tg $\epsilon 26$ mice are of donor origin. B cells (B220⁺; clone RA3-6B2) and T cells (α/β TCR⁺, clone H57-597) from lymph nodes of donor mice (H-2^{b/k}; (C57BL/6 $\times$ CBA/J)F₁), naive and bone-marrow-transplanted tg $\epsilon 26$ animals (H-2^k) were stained 5 weeks after transplantation for surface expression of H-2K^b (clone AF6-88.5; FITC labelled) and H-2K^k (clone AF3-12.1, phycoerythrin(PE) labelled). Three-colour analysis was performed by flow cytometry with electronic gating for α/β TCR⁺ or B220⁺ cells using streptavidin-labelled Red 670 (Gibco/BRL) for the detection of the different lymphoid populations. **b**, **c**, T cells from transplanted tg $\epsilon 26$ mice are functionally impaired in their response to allogeneic cells. Mixed lymphocyte reaction (MLR; **a**) and cytotoxic T lymphocyte (CTL; **b**) responses from tg $\epsilon 26$ mice (circles), (C57BL/6 $\times$ CBA/J)F₁ (squares), bone marrow (C57BL/6 $\times$ CBA/J)F₁ reconstituted tg $\epsilon 26$ (triangles) and RAG-2^{-/-} (diamonds) mice. METHODS. Six weeks after transplantation, purified T cells from lymph node of naive tg $\epsilon 26$ mice, from (C57BL/6 $\times$ CBA/J)F₁ mice and from (C57BL/6 $\times$ CBA/J)F₁ bone-marrow-transplanted RAG-2^{-/-} and tg $\epsilon 26$ animals were co-cultured with irradiated BALB/c splenocyte. For MLR, proliferation of 2×10^5 cells was measured after 5 days by incorporation of [³H]-thymidine during the last 18 h. For the CTL response, cytotoxicity was measured in a 4-h chromium release assay using P815 target cells. The percentage of specific lysis has been normalized to represent equal amounts of T-cell per target ratios.



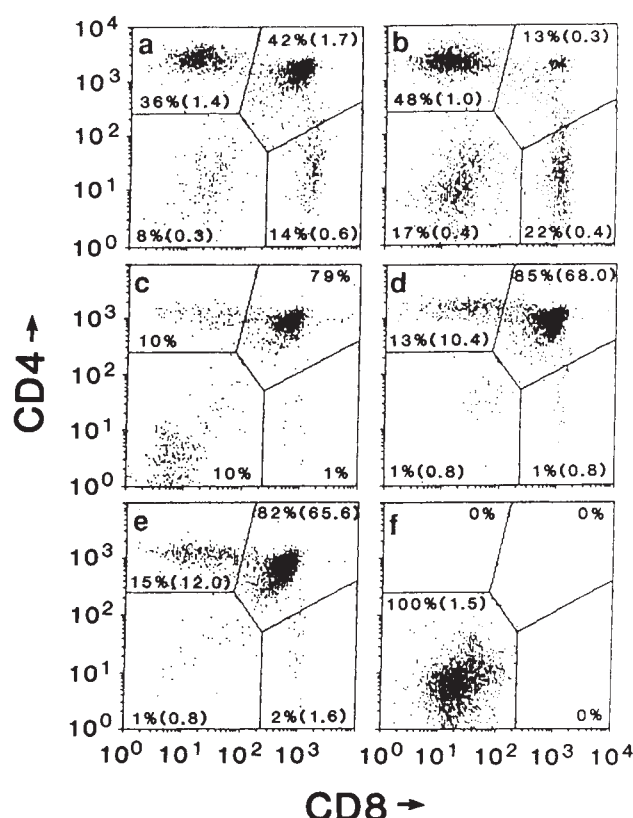


FIG. 3 Thymocyte development in transplanted tg $\epsilon 26$ mice. Thymocytes were analysed by flow cytometry for the surface expression of CD4 (PE-labelled, clone RM4-4) and CD8 (FITC-labelled; clone 53-6.7). The relative (%) and absolute ($\times 10^6$) number of cells are given for each subpopulation of thymocytes. Thymic tissue was obtained from (C57BL/6 $\times$ CBA/J) F_1 bone-marrow-reconstituted tg $\epsilon 26$ mice 4.5 (a) and 7 weeks (b) after transplantation; c, from fetal day 16 tg $\epsilon 26$ thymus plus (C57BL/6 $\times$ CBA/J) F_1 bone-marrow-transplanted adult tg $\epsilon 26$ mice; d, from tg $\epsilon 26$ mice reconstituted *in utero* with congenic C57BL/6Thy-1 a lgM a haematopoietic precursor cells; e, from RAG-2 $^{-/-}$ mice transplanted with (C57BL/6 $\times$ CBA/J) F_1 bone marrow; and f, from naive tg $\epsilon 26$ animals.

($\sim 8 \times 10^7$ cells) of these tg $\epsilon 26$ mice appeared to be normal as judged by immunohistology (Fig. 1A, g, h). The distribution of the four thymocyte subsets was identical to that found in reconstituted RAG-2 $^{-/-}$ mice (Fig. 3d, e) and the peripheral T lymphocytes were also phenotypically (Table 1) and functionally normal. Taken together, these experiments showed that the thymic stroma of tg $\epsilon 26$ mice could differentiate into cortical and medullary domains and so support normal T-lymphocyte differentiation. As normal thymic differentiation could be rescued in fetal but not in adult tg $\epsilon 26$ thymuses, our results suggest that the induction of a normal cortical microenvironment takes place within a defined period in early development.

Our results provide strong evidence that T-cell precursors between the early developmental stages of CD44 $^+$ CD25 $^-$ and CD44 $^-$ CD25 $^+$ (ref. 25) are able to effect the development of the thymus anlage into cortex and medulla. The ability to induce a normal cortical microenvironment was comparable for fetal and adult haematopoietic precursor cells. Further evidence consistent with the idea that precursor thymocytes influence stromal development comes from certain forms of human severe combined immunodeficiency syndromes: thymic tissue from these patients consists of a relatively dense and undifferentiated thymic epithelium without a recognizable separation into cortex and medulla and completely lacks lymphoid cells 26 . □

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CD8 modulation of T-cell antigen receptor–ligand interactions on living cytotoxic T lymphocytes

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THYMOCYTES and class I major histocompatibility complex (MHC)-restricted cytotoxic T lymphocytes express predominantly heterodimeric $\alpha\beta$ CD8 1,2 . By interacting with non-polymorphic regions of MHC class I molecules CD8 can mediate adhesion $^{3-6}$ or by binding the same MHC molecules that interact with the T-cell antigen receptor (TCR) function as coreceptor in TCR–ligand binding and T-cell activation 1,2 . Using TCR photoaffinity labelling with a soluble, monomeric photoreactive H-2K d -peptide derivative complex 7 , we report here that the avidity of TCR–ligand interactions on cloned cytotoxic T cells is very greatly strengthened by CD8. This is primarily explained by coordinate binding of ligand molecules by CD8 and TCR, because substitution of Asp 227 of K d with Lys severely impaired the TCR–ligand binding on CD8 $^+$, but not CD8 $^-$ cells. Kinetic studies on CD8 $^+$ and CD8 $^-$ cells further showed that CD8 imposes distinct dynamics and a remarkable temperature dependence on TCR–ligand interactions. We propose that the ability of CD8 to act as coreceptor can be modulated by CD8–TCR interactions.

To assess TCR–ligand interactions by TCR photoaffinity labelling 7 the K d -restricted *Plasmodium berghei* circumsporozoite peptide PbCS 253–260 (single-letter notation, YIPSAEKI) was N-terminally conjugated with photoreactive iodo, 4-azido-

salicylic acid (IASA) and at the TCR contact residue Lys 259 with 4-azidobenzoic acid (ABA). Selective photoactivation of the IASA group allowed the covalent attachment of this conjugate to soluble, monomeric K^d molecules (K^dQ10). Incubation of these complexes with cloned, [IASA]-YIPSAEK[ABA]I-reactive T1 cytotoxic T cells and photoactivation of the ABA group resulted in TCR photoaffinity labelling (Fig. 1a, e). The TCR-ligand interaction on which this labelling was based, involved, as well as specific K^d -TCR contacts, an intimate contact of the photoreactive ligand side chain with defined $V\beta 8.2$ - and $J\alpha 20$ -encoded TCR residues (I.F.L. *et al.*, manuscript in preparation). This labelling was completely inhibited by the anti- K^d monoclonal antibody 20-8-4S, which blocks specific TCR-ligand interactions⁷, but not by the anti-LFA-1 monoclonal antibody FD18.5 (Fig. 1a, e). In contrast, the TCR photoaffinity labelling was reduced by over 95% in the presence of the anti-CD8 β monoclonal antibody H35-17 and 53.5.81 and F(ab') fragments of the anti- $K^d\alpha 3$ monoclonal antibody SF1.111 to levels observed on CD8⁻, T1 cytotoxic T lymphocyte (CTL)-derived T1.4 cells (Fig. 1b). Transfection of T1.4 cells with CD8 α partially restored the efficient ligand binding (Fig. 1c), indicating that these considerable differences in TCR-ligand binding are accounted for by CD8. This CD8 transfectant, 16.3.2, expressed about three times higher levels of CD8 α than T1 cytotoxic T cells, but roughly five times lower levels of CD8 β (see legend to Fig. 1). Thus, although T1 cytotoxic T cells expressed predominantly CD8 α/β , 16.3.2 cells apparently expressed both CD8 α/β

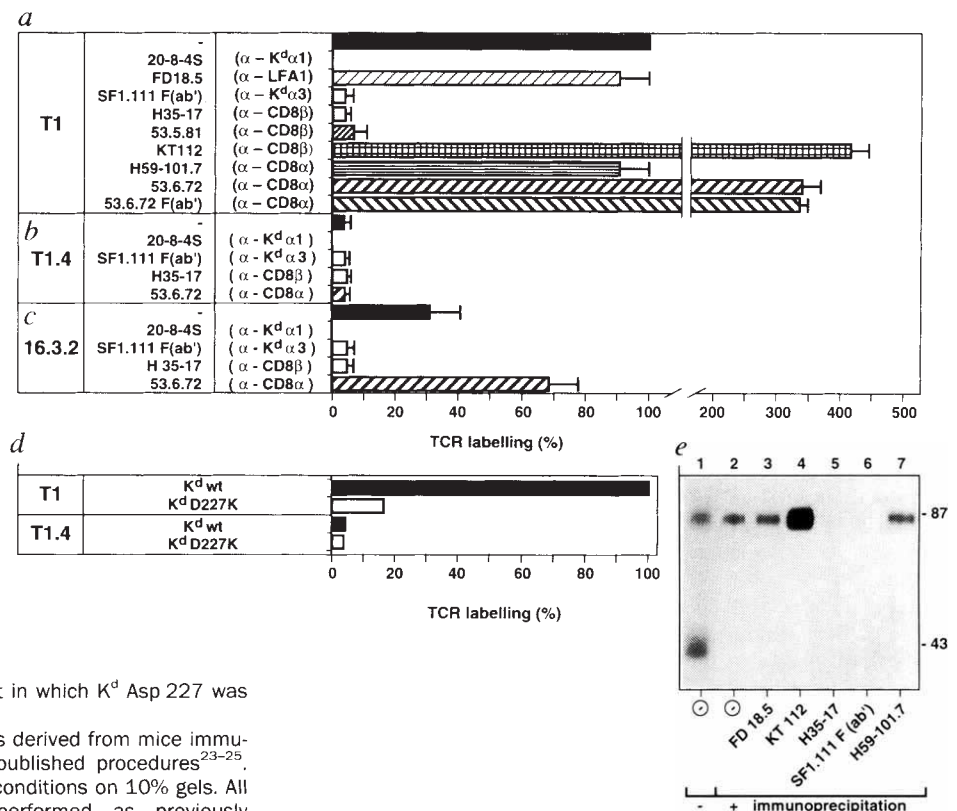
and CD8 α/α molecules. As all three cell types expressed comparable levels of TCR and CD3, the correlation of the increase in TCR-ligand binding with CD8 α/β expression suggests that CD8 α/β may be more efficient than CD8 α/α in supporting the TCR-ligand binding.

In the presence of either the anti-CD8 β monoclonal antibody KT112 or the anti-CD8 α antibody 53.6.72 the TCR-ligand binding was increased about three- or fourfold (Fig. 1a). This was also observed at 0–4 °C (not shown). As F(ab') fragments of 53.6.72 were equally active (Fig. 1a), and these anti-CD8 antibodies had no effect on the TCR-ligand binding on T1.4 cells (Fig. 1b and data not shown), it seems that CD8 crosslinking or involvement of Fc receptors are not responsible for these effects, which may instead be related to antibody-induced conformational changes in CD8.

Substitution of K^d Asp 227 with Lys in the soluble ligand resulted in a more than 80% reduction of the TCR labelling on T1 cytotoxic T cells, but had no detectable effect on the labelling on T1.4 cells (Fig. 1d). As Asp 227 is critical for CD8-MHC class I interactions⁸, these findings show that CD8 can interact with TCR-bound MHC class I molecules and that this interaction results in a substantial increase of the overall ligand binding avidity. Similar results were obtained with other [IASA]-YIPSAEK[ABA]I-specific clones of cytotoxic T cells, whose antigen recognition was CD8-dependent to different degrees (ref. 7 and unpublished data). These findings are novel in that CD4

FIG. 1. Binding of monomeric soluble K^d -peptide derivative complexes on living cells assessed by TCR photoaffinity labelling. a, T1 cytotoxic T cells were incubated with K^dQ10 photoaffinity labelled beforehand with [¹²⁵I]IASA-YIPSAEK[ABA]I (ref. 7), at 37 °C for 30 min in the absence or presence of the indicated antibody (5 μ g ml⁻¹) or F(ab') fragments (10 μ g ml⁻¹). After ultraviolet (UV) irradiation the total cell lysate or the immunoprecipitates with anti-TCR antibody H57-597 were analysed by SDS-PAGE (e). The amounts of covalent trimolecular complexes were quantified by densitometric analysis of autoradiograms. The CD8⁻ T1.4 hybridoma (b) and its corresponding CD8 α transfectant 16.3.2 (c) were tested likewise. The amount of photoaffinity-labelled TCR observed on the T1 cytotoxic T cells in the absence of antibody was defined as 100%. The standard deviations were calculated from three different experiments according to the Student *t* method. Alternatively, T1 cytotoxic T cells and T1.4 cells were tested likewise with soluble wild-type K^d -peptide derivative complex (K^d WT) or with a variant in which K^d Asp 227 was substituted with Lys (K^d D227K) (d).

METHODS. The T1 cytotoxic T-cell clone was derived from mice immunized with [IASA]-YIPSAEK[ABA]I following published procedures^{23–25}. SDS-PAGE was performed under reducing conditions on 10% gels. All labelling procedures were essentially performed as previously described⁷. In brief, the cells (1.5×10^5 in 500 μ l) were preincubated in 12-well plates with the purified anti-CD8 β antibody H35-17²⁶ or 53.5.81²⁷ or KT112²⁸ or anti-CD8 α antibody 53.6.72 (American Type Culture Collection (ATCC), Rockville, MD) or H59-101.7²⁶ or anti-LFA-1 antibody FD18.5⁷ or anti- K^d antibody 20-8-4S (ref. 7 and ATCC) or SF1.111 (ref. 7 and ATCC) F(ab') fragments at 37 °C for 15 min before addition of monomeric, purified K^dQ10 ~[¹²⁵I]IASA-YIPSAEK[ABA]I (3×10^6 c.p.m., corresponding to 3×10^{-9} M final concentration). The T1.4 hybridoma cells were derived by fusion of T1 cytotoxic T cells with the BW5147 $\alpha\beta$ thymoma. In this type of T-cell hybrid, the BW5147 thymoma contributes transacting negative factors, which repress the



expression of the incoming CD8 α but not CD8 β gene²⁹. Transfection of the T1.4 hybridoma with CD8 α cDNA and selection in the presence of G418-sulphate were performed as described³⁰. When analysed by flow cytometry, the resulting 16.3.2 cells displayed mean fluorescence of 315 for CD8 α and 17 for CD8 β (the background was 2.8 and 3.0, respectively). Under identical conditions T1 cytotoxic T cells showed a mean fluorescence of 96 for CD8 α and 83 for CD8 β . T1 cytotoxic T cells, T1.4 and 16.3.2 cells expressed comparable levels ($\pm \leq 12\%$) of CD3 and TCR, as assessed by flow cytometry following staining with antibodies 145.2 C11 and H57-597 respectively (ATCC).

and CD8 have so far been reported not to contribute significantly to the TCR–ligand binding^{9,10}.

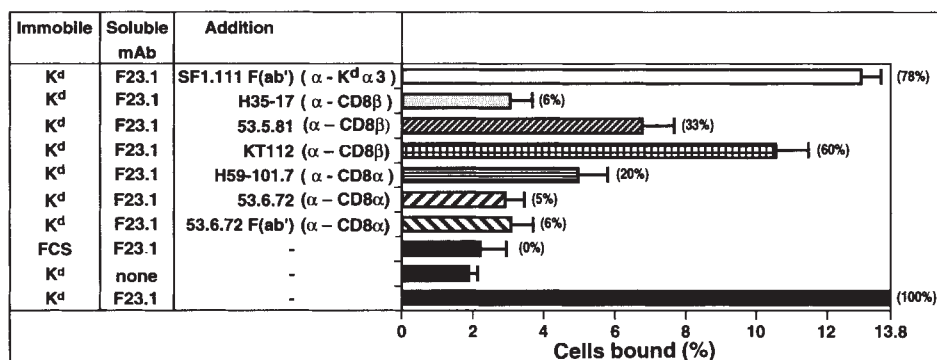
Others have shown that CD8-dependent adhesion of cytotoxic T cells to immobilized MHC class I molecules can be induced by low concentrations of soluble anti-TCR antibodies^{5,6}. Using this assay we found that the adhesion of T1 cytotoxic T cells to immobilized K^d molecules was strongly inhibited by the anti-CD8 β antibody H35-17 (Fig. 2), which also inhibited the TCR–ligand binding (Fig. 1b). All the other reagents tested, however, had different effects in these two assays (compare Figs 1b and 2). For example, the anti-CD8 α antibody H59-101.7 barely affected the TCR–ligand binding, but considerably inhibited the adhesion of T1 cytotoxic T cells. Similar results were obtained with several other anti-CD8 α antibodies (data not shown). On the other hand, of the anti-CD8 β antibodies tested, all substantially interfered with the TCR–ligand binding (Fig. 1a and data not shown) and CD8 α/β seems to support the TCR–ligand binding more efficiently than CD8 α/α ; it is therefore tempting to speculate that CD8–MHC class I interactions relevant for the CD8 coreceptor function may involve, at least in part, CD8 β . This may explain the importance of CD8 β in T-cell

development¹¹ and antigen recognition^{12,13} even though CD8 β , unlike CD8 α , according to current knowledge, is not directly involved in signalling^{1,2,14,15}.

Kinetic experiments showed that the TCR–ligand binding on T1 cytotoxic T cells was remarkably temperature-dependent, and biphasic at 37 °C and 18 °C, but not at 0 °C (Fig. 3a). At 37 °C a transient binding maximum was reached after 6 min, followed by an about 50% decrease in the next 10–15 min. At 18 °C, in a similar binding pattern, a transient binding maximum was reached after ~40 min. In contrast, at 0 °C the ligand binding continuously increased over 3 h, to reach a stable binding maximum after 3–4 h. The maximal binding at 0 °C was about 30% and 75% higher than at 18 °C and 37 °C, respectively. The dissociation of TCR–ligand complexes on T1 cytotoxic T cells was similarly temperature dependent (Fig. 3b). At 37 °C 50% of the complexes were dissociated after ~90 s, at 18 °C after 7 min and at 0 °C after 30 min. The amount of immunoprecipitable photoaffinity-labelled TCR decreased less than 10% during 80 min of incubation at 37 °C (Fig. 3b dotted line). This decrease may be accounted for by TCR endocytosis and possibly intracellular degradation of TCR–ligand complexes¹⁶. Very similar binding

FIG. 2 Anti-TCR antibody activated, CD8-mediated adhesion of T1 cytotoxic T cells. ⁵¹Cr-labelled T1 cells were incubated in 96-well plates onto which K^d was previously immobilized (0.25 μ g per well) in the absence or presence of the indicated reagents (same concentrations as in Fig. 1). The adhesion was induced by addition of anti-TCR F23.1 (0.25 μ g ml⁻¹). The percentages shown on the bars indicate the inhibition of the specific T1 cell adhesion brought about by the different reagents.

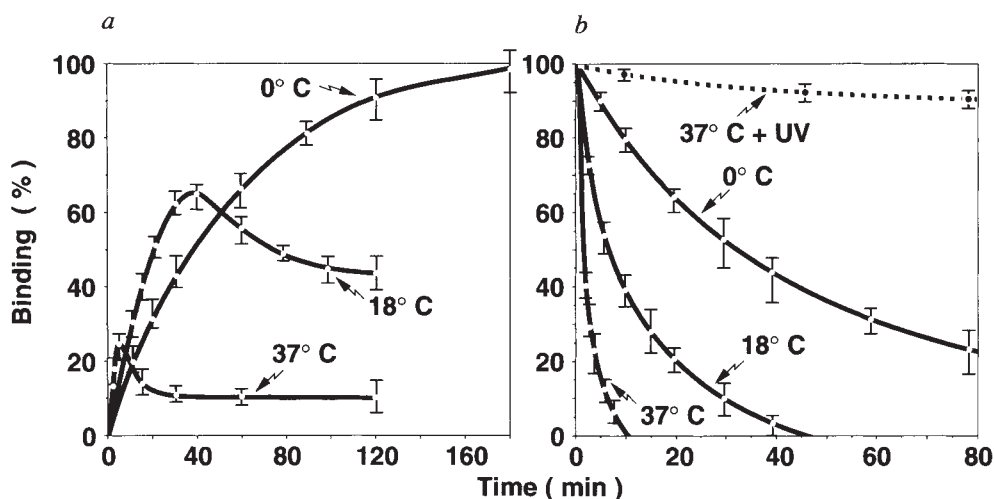
METHODS. The anti-TCR antibody induced, CD8-dependent adhesion assay was performed as described⁵, except that MEGA 8 (70 mM) was used as detergent instead of deoxycholate. The representative experiment shown was performed in triplicate. Binding was calculated as percentage cells bound = 100 (c.p.m. bound)/(total c.p.m. – spontaneous



c.p.m.)). In this experiment total ⁵¹Cr incorporation was 61,838 c.p.m. per 10⁵ cells and spontaneous release was 1,856 c.p.m. The incubation time was 2 h 15 min.

FIG. 3 Kinetics of the TCR–ligand interactions on T1 cytotoxic T cells. **a**, For association kinetics T1 cytotoxic T cells were incubated with K^dQ10 ~ [125I]ASA-YIPSEAK[ABA] as indicated. After UV irradiation the immunoprecipitated trimolecular complexes were quantified as described for Fig. 1. Hundred per cent binding corresponds to maximal binding reached at 0 °C. **b**, For dissociation kinetics T1 cytotoxic T cells were preincubated with K^dQ10 ~ [125I]ASA-YIPSEAK[ABA] and at time 0 diluted 21-fold in medium containing the anti-K^d antibody 20-8-4S to prevent ligand rebinding. Aliquots were UV irradiated at various times and the amounts of trimolecular complexes quantified. Alternatively, the incubation mixture was UV irradiated at time 0 (dotted line).

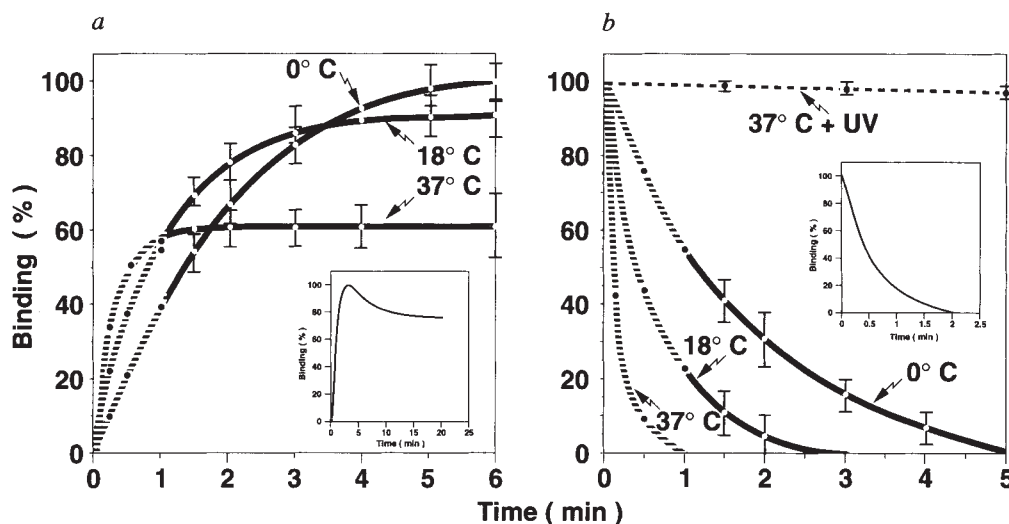
METHODS. The association experiments were performed as described for Fig. 1 by using the specified incubation conditions. For dissociation experiments, the cells (2 × 10⁷ ml⁻¹) were incubated with K^dQ10 peptide derivative complex (2 × 10⁷ c.p.m. ml⁻¹) at 0 °C for 2 h. Fifty micro-



litre samples of this incubation mixture were added into Petri dishes (3 cm diameter) containing 1 ml equilibrated medium and 10 μ g 20-8-4S antibody. The data from four independent experiments were used for calculating means and standard deviations.

FIG. 4 Kinetics of TCR–ligand interactions on T1.4 cells. The association (a) and dissociation (b) kinetics were assessed on T1.4 cells as described for Fig. 3. The accuracy of the binding values for time <1 min (dotted lines), is limited because of the time required for UV irradiation (15 s). One hundred per cent binding corresponds to maximal binding reached at 0 °C. Alternatively, as shown in the inserts, the same experiments were performed on 16.3.2 cells at 37 °C.

METHODS. As described for Fig. 3. For dissociation experiments T1.4 cells were preincubated with soluble ligand at 0 °C for >10 min. Means and standard deviations were calculated from three independent experiments.



patterns have been observed on another [IASA]-YIPSAEK[ABA]I-reactive cytotoxic T-cell clone⁷.

Remarkably different results were obtained when the same kinetic experiments were performed on CD8⁺ T1.4 cells (Fig. 4). Here the ligand binding was monophasic at all three temperatures and maximal binding was reached after ~1 min at 37 °C, 3–4 min at 18 °C and 5–6 min at 0 °C (Fig. 4a). The maximal degree of binding reached at 37 °C and 18 °C were about 22% and 8% lower than at 0 °C. The dissociation of TCR–ligand complexes was similarly different. At 37 °C 50% of the complexes dissociated on T1.4 cells after ~7 s, at 18 °C after ~20 s and at 0 °C after 70 s (Fig. 4b). Whereas essentially the same association and dissociation kinetics were observed on T1 cytotoxic T cells in the presence of SF1.111 F(ab') (data not shown), those measured on 16.3.2 cells were clearly different, that is, shifted towards those observed on T1 cytotoxic T cells (Fig. 4 inserts). Taken collectively these results indicate that these considerably different binding patterns are essentially accounted for by CD8.

The remarkable temperature dependence and the biphasic nature of the ligand binding observed on T1 cytotoxic T cells (Fig. 3) are not explained by ligand-induced variation of the expression of CD8 or CD3/TCR, or by depletion of ligand by CD8 (unpublished observations). It thus seems that the ligand binding modulates the participation of CD8 in TCR–ligand interactions in a time- and temperature-dependent manner. CD8 and CD4 have been reported to interact physically with the TCR^{17–19}. The observation that F(ab') fragments of SF1.111 and 53.6.72 monoclonal antibody and other reagents affected the TCR–ligand binding and adhesion of T1 cytotoxic T cells differently, whereas substitution of Asp 227 with Lys in K^d similarly inhibited both CD8 functions (Figs 1 and 2 and ref. 8) suggests that such CD8–TCR interactions are also relevant in the present system. These interactions may be critical for the modulation of the CD8 participation in the TCR–ligand binding. It is conceivable that these interactions define a conformational state and an orientation of CD8 relative to the TCR, which determine its ability to cooperate with the TCR in the ligand binding. CD8 also has been shown to interact with other entities, such as CD3²⁰, CD45²¹ or intracellularly with p56^{lck} tyrosine kinase²². These species, as well as CD8 itself, are intimately involved in cellular processes of antigen-specific cell activation, which are initiated by TCR–ligand interactions^{1,2,14}. Many of these processes are metabolically active and involve structural changes in these molecules. It is therefore possible that TCR–ligand binding

affects, by such processes, CD8–TCR interactions and hence the ability of CD8 to act as coreceptor. It is interesting to note that reagents of widely different reactivity, such as the anti-CD8 β antibody KT112 or the anti-CD45 antibody B220, and also the protein kinase inhibitor staurosporin very significantly affect the degree and the dynamics of TCR–ligand interactions on T1 cytotoxic T cells (unpublished observation). Taken collectively, these observations indicate that the participation of CD8 in the TCR–ligand binding is very significant and subject to modulation through complex molecular and cellular processes. □

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Neisseria PilC protein identified as type-4 pilus tip-located adhesin

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TYPE-4 pilus-mediated adherence of *Neisseria gonorrhoeae* and *Neisseria meningitidis* is considered to be a crucial early event in neisserial infections^{1,2}. In addition to the principal pilus subunit (pilin or PilE), both pathogens produce low quantities of a phase-variable PilC protein which is implicated in pilus biogenesis and pilus-mediated epithelial cell adherence^{3,4}. The identity, however, of the pilus adhesin has remained obscure^{4,5}. Here we describe the isolation of a PilC protein from a gonococcal overproducing strain and demonstrate its specific interaction with human epithelial cells. Our results are consistent with the cell and species tropisms of neisserial infections. Binding of PilC effectively competes with pilus-mediated, but not Opa-mediated⁶, attachment of *N. gonorrhoeae* and of *N. meningitidis*, indicating that both pathogens interact with identical or very similar epithelial cell receptors. Immunogold electron microscopy using antisera raised against purified PilC and synthetic peptides locates PilC at the tip of gonococcal pili. PilC thus represents an essential pilus-associated adhesin, providing a rationale for selective protection against neisserial infections.

PilC protein was purified to homogeneity (>98%) from a gonococcal overproducing PilE-deficient strain (Fig. 1) and used to assess its interaction with various human and non-human cell types. Consistent with the species specificity of neisserial infections, purified PilC bound to human epithelial cells such as ME-180 human cervix carcinoma cells (Fig. 2b). But it did not bind to fibroblasts or epithelial cells of non-human origin, for example MDCK (Fig. 2c), MDBK, PSEK or NIH3T3 cells (data not shown). Purified PilC protein showed a strong tropism for primary epithelial cells from human cornea rather than epithelial cells from sheep, porcine or bovine cornea explants (data not shown). To assess whether the binding of purified PilC competitively blocked adherence of piliated gonococci, ME-180 cells were preincubated with purified PilC protein and subsequently infected. Attachment of strain MS11-N138 (P⁺, PilE_{F3})⁴, which adheres strongly to ME-180 cells (Fig. 2d), was completely inhibited after pretreatment of the target cells with PilC (Fig. 2e). MDCK cells that do not bind PilC do not bind gonococci either (Fig. 2c, f). Competition experiments with defined variants of *N. gonorrhoeae* MS11 producing either PilC1 (N558) or PilC2 (N557) gave similar results when assayed with three different human epithelial cell lines, ME-180, Hec-1B and RT112 (Table 1). Inhibition was independent of PilE variation (compare variants N137 and N138). Moreover, binding of PilC_{2His6} to ME-180 cells also blocked the adherence of two *N. meningitidis* isolates producing different classes of type-4 pili (Table 1 and Fig. 2g-i). By contrast, Opa-mediated adherence, representing a subsequent step in neisserial infections⁶, was not inhibited by PilC (Fig. 2j-l). In addition, pilus-dependent agglutination of human erythrocytes was not inhibited (data not shown). Heat-treated PilC_{2His6}, buffer without PilC, and an unrelated His₆-tagged antibody fragment (FP11)⁷ did not inhibit neisserial attachment (Table 1). These data clearly demonstrate competition of purified PilC_{2His6} with P⁺ organisms of both pathogenic *Neisseria* species for a distinct receptor present on human epithelial cells.

Immunogold electron microscopy using antisera directed against PilC1 or PilC2 revealed a specific labelling of the thin (~6 nm diameter) and very fragile *N. gonorrhoeae* type-4 pili (Fig. 3a, c). At high magnifications, almost all detectable gold particles and electron-dense immune complexes are associated with the tips of pilus fibres (Fig. 3b, e). In some instances, tip-labelled pili were still anchored in the bacterial cell surface

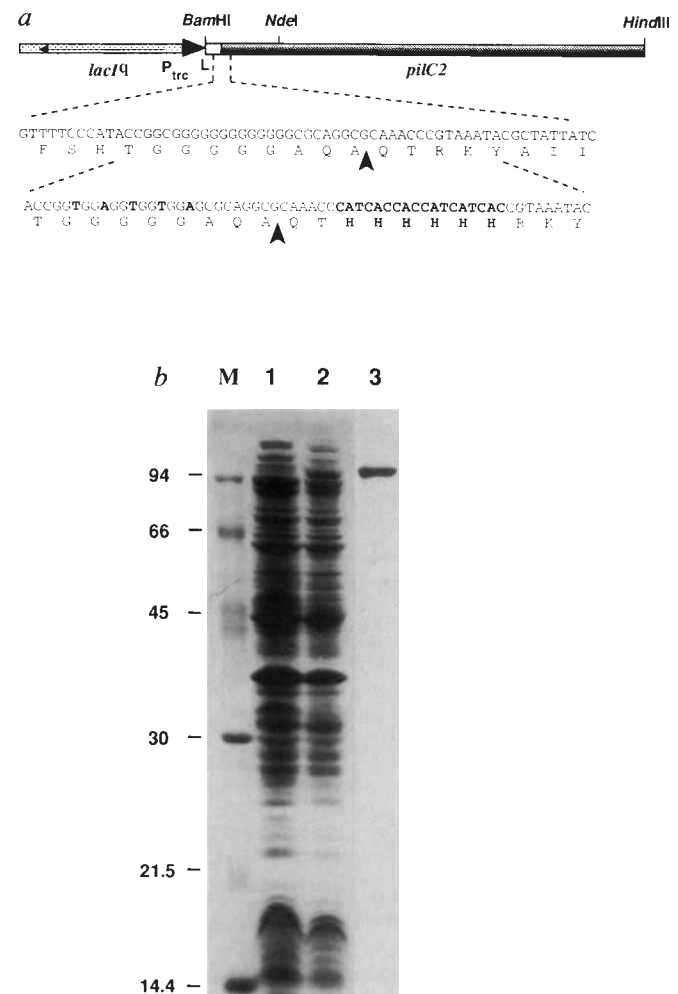


FIG. 1 Overproduction and isolation of *N. gonorrhoeae* MS11 PilC_{2His6} protein. **a**, Structure of an invariant *pilC2* gene of strain MS11 containing a His₆ tag-encoding sequence. Modification of the variable poly(G) run³ in the signal-peptide encoding region eliminated the variability of *pilC2* expression. Insertion of the His₆ tag-encoding sequence immediately downstream of the signal peptide cleavage site (arrowhead) allowed efficient purification of recombinant PilC₂ protein. The modified *pilC2* gene was under the control of the *P_{trc}* promoter and expressed in *N. gonorrhoeae* N560 in the presence of IPTG. The wild-type (top sequence) and modified (bottom sequence) sequences of *pilC2* are shown. **b**, Protein gels demonstrating overproduction and purification of PilC_{2His6}. Lanes 1 and 2, Coomassie-blue-stained lysates of strain N560 non-induced and IPTG-induced producing large amounts of PilC, respectively. Lane 3, silver-stained gel of affinity-purified PilC protein. M, M_r markers.

METHODS. Modifications were introduced into the cloned *pilC2* gene of strain MS11 using a PCR strategy described elsewhere (T.R. *et al.*, manuscript in preparation). The modified *pilC2*_{His6} gene was inserted into the shuttle vector Hermes-8 (E.-M. Kupsch *et al.*, manuscript in preparation), used to transform N219 and subsequently transconjugated into N431 (both strains ref. 4), a P⁻, Δ *pilE* derivative of *N. gonorrhoeae* MS11, to yield N560. This strain was devoid of PilE and allowed purification of the recombinant PilC_{2His6} protein from lysates by NTA affinity chromatography²⁶.

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(Fig. 3e). The PilC2-specific serum labelled tip-located PilC2 protein independently of the PilE protein variant contained within the pili (Fig. 3e). No labelling of the pili was seen with strain N556 (*pilC1,2*), which produces neither PilC1 nor PilC2 (Fig. 3d). Although we saw the occasional association of PilC along the pilus fibres, these experiments showed that PilC was located at the tip of the gonococcal pili.

Type-4 pili are produced by a variety of important human bacterial pathogens, including *Pseudomonas aeruginosa*⁸, enteropathogenic and enterotoxigenic *Escherichia coli* (EPEC/ETEC)^{9,10}, and *Vibrio cholerae*^{11,12}. These pili differ substantially with regard to subunit structure, diameter and assembly process from the well studied *E. coli* Pap pili and their homologues, which mostly have tip-located adhesins¹³. The existence of minor subunits in the *N. gonorrhoeae* pili has been proposed^{14–16}, but we have shown here that PilC is essential as a tip-located adhesin. It is not known whether the heteropolymeric nature of the neisserial pili applies to other species of type-4 pilus. A role for type-4 pilin as an adhesin has been demonstrated in *Pseudomonas aeruginosa*¹⁷. However, the *Neisseria* pili are noted for intrastrain variability of the PilE subunit^{18–20}, which may account for the relative conservation of PilC proteins in order to maintain the potential of pili to interact with a conserved receptor²¹.

Pilus-dependent attachment of neisserial strains to epithelial cells is influenced by PilE subunit variation^{4,22,23}. A correlation has been proposed between the variant-specific glycosylation of PilE and cell adherence²⁴. In the context of our findings we assume that PilE variation indirectly affects the adherence function of PilC, for example through variant-specific glycosylation of the pilus. Earlier work has suggested a direct role of PilE in pilus-mediated attachment of *N. gonorrhoeae* to human epithelial cells²⁵. This does not necessarily conflict with our data as gonococcal pili have at least one additional receptor-binding property which is independent of PilC and detectable by the agglutination of human erythrocytes⁴. Our results indicate that PilC-mediated epithelial cell interaction is crucial for mucosal cell infections and is a key target for preventing meningococcal meningitis and venereal disease. Using PilC-specific antisera, we

TABLE 1 Inhibition of epithelial cell attachment of pathogenic *Neisseria* species by purified PilC protein

Strains (relevant phenotypes)	Adherence of <i>Neisseria</i> species to epithelial cells	
	Without PilC	With PilC
	ME-180	
Ngo N137 (P ⁺ , PilE _{E1})	++	–
Ngo N138 (P ⁺ , PilE _{F3})	++	–
Ngo N138 (P ⁺ , PilE _{F3})		++*
Ngo N138 (P ⁺ , PilE _{F3})		+++†
Ngo N558 (P ⁺ , PilE _{E1} , PilC1)	++	–
Ngo N557 (P ⁺ , PilE _{E1} , PilC2)	++	–
Ngo N556 (P ⁺ , PilC [–])	–	–
Ngo N431 (P ⁺ , PilE [–] , PilC ⁺)	–	–
Nme N530 B380 (P ⁺ , class IIa/IIb)	+	–
Nme N559 Z4025 (P ⁺ , class I/IIa)	++	–
	Hec-1B	
Ngo N137 (P ⁺ , PilE _{E1})	++	–
Ngo N138 (P ⁺ , PilE _{F3})	++	–
Nme N559 Z4025 (P ⁺ , class I/IIa)	++	–
	RT112	
Ngo N137 (P ⁺ , PilE _{E1})	++	–
Ngo N138 (P ⁺ , PilE _{F3})	++	–

Adherence and adherence-competition experiments were performed as described (Fig. 2); (–) <5, (+) 25–50, (++) >50 adherent bacteria per epithelial cell. Epithelial cell lines: ME-180 human cervix carcinoma (ATCC HTB33), Hec-1B human endometrium carcinoma (ATCC HTB133), RT112 human bladder carcinoma (provided by W. Franke). The MDCK canine kidney (ATCC CCL34), MDBK bovine and PSEK porcine kidney (both provided by H.-J. Rziha), mouse NIH3T3 fibroblast (ATCC CRL1658) cell lines were used in other experiments. All strains except N556, N557, N558 (unpublished) and N530, N559 (provided by M. Achtman²⁷), have been described⁴. Strains N530 and N559 were selected for piliation (P⁺) by pellicle formation before analysis in this assay. Ngo, *Neisseria gonorrhoeae*; Nme, *Neisseria meningitidis*.

* PilC protein was boiled for 2 min before being applied to epithelial cells.

† Epithelial cells were preincubated with a control eluate containing no PilC.

FIG. 2 Adherence of PilC to epithelial cells and competition with neisserial attachment. a–d, Binding of purified PilC to human ME-180 epithelial cells (b) and canine MDCK epithelial cells (c) developed with anti-PilC serum (AK199) and FITC-labelled secondary antibodies; control experiment with ME-180 cells in the absence of PilC (a). d–f, Strain N138 adheres strongly to ME-180 cells (d) but not to MDCK cells (f); preincubation of the epithelial cells with purified PilC_{His6} inhibits attachment of N138 (P⁺) to ME-180 cells (e). g–i, Like (d–f) but using *N. meningitidis* N559 (P⁺). j–l, Like (d–f) but using *N. gonorrhoeae* strain N303 (PilE[–], Opa₅₀⁺). For additional data, see Table 1. Heat-inactivated PilC_{His6} or an unrelated His₆-tagged light-chain antibody fragment (FP11) showed no signs of binding to any of the tested cells, excluding an interaction of the His₆ tag with epithelial cells.

METHODS. For direct binding of PilC, purified PilC (2 µg ml^{–1}) was incubated with target cells for 30 min at 37 °C and removed by extensive washing with PBS. Cells and bound PilC were fixed with 2% paraformaldehyde in PBS for 15 min at room temperature and incubated in 400-fold dilutions of anti-PilC serum AK199. PilC-antibody complexes were stained with FITC-labelled secondary antibodies directed against anti-mouse IgG and labelled cells were viewed under a Zeiss Axiovert 35 microscope. Target cells were preincubated with purified PilC (2 µg ml^{–1}) at 37 °C for 30 min and infected with 2 × 10⁷ bacteria for 1 h at 37 °C and 5% CO₂. The P[–] Opa₅₀⁺ strain was grown in defined liquid medium and gently centrifuged onto target cells before the 1-h incubation period. After washing and fixation with 2% paraformaldehyde in PBS, infected cells and bacteria were stained with crystal violet.

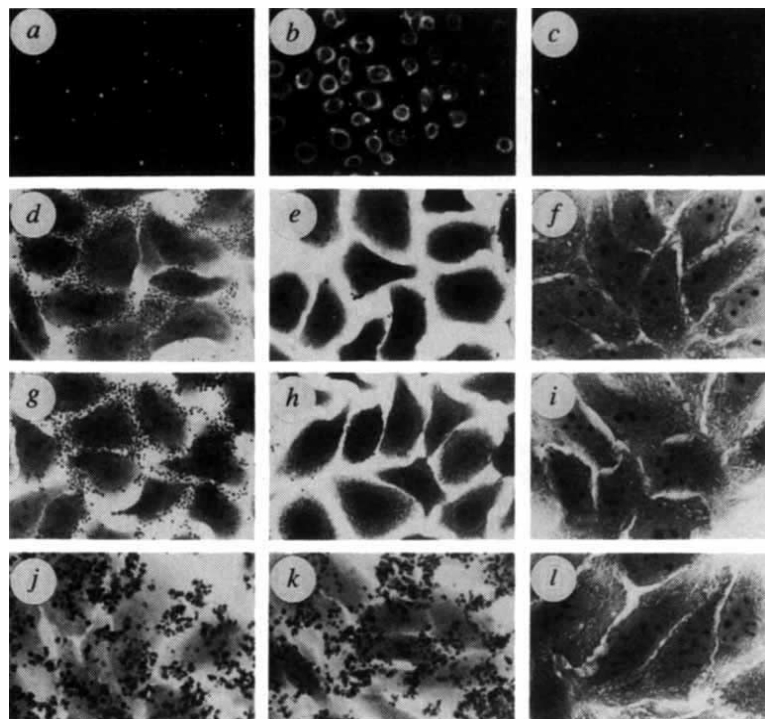
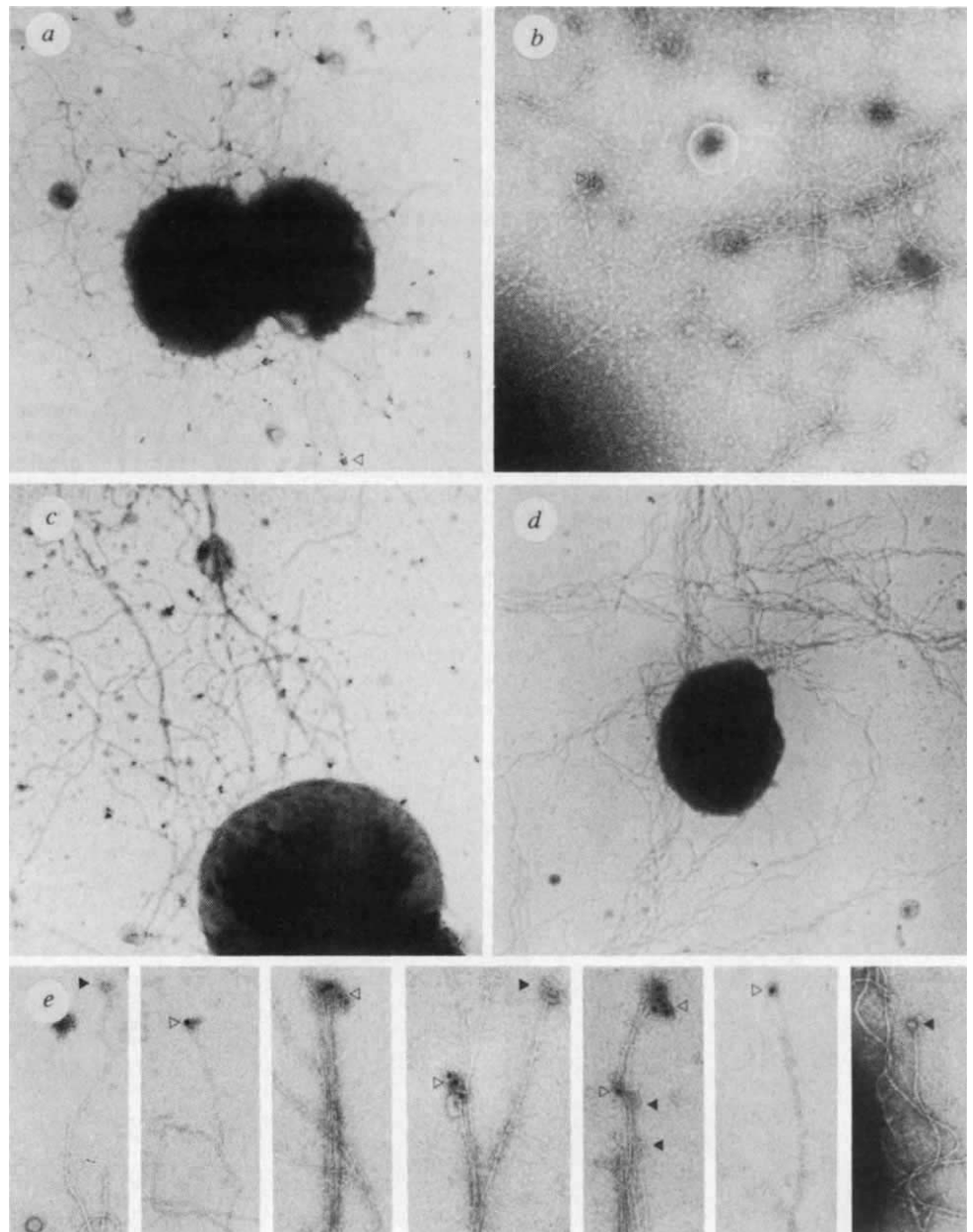


FIG. 3 Location of PilC in the pili of *N. gonorrhoeae* by immunogold electron microscopy. Strain N137 (PilC²) reacted with anti-PilC_{His6} serum AK199 (a, b) and strain N473 (PilC¹) reacted with anti-peptide PilC1 serum AK200 (c). No label can be seen on strain N556 (PilC⁻) using AK200 antiserum (d). Details showing tip-labelled pili of strains N137 or N138 (both PilC²) after incubation with AK199 (e). Photographs were taken at $\times 12,000$ (a, d), $\times 18,000$ (c) or $\times 36,000$ (b, e) magnification. Open triangles indicate gold particles; filled triangles indicate stained immune complexes of primary and secondary antibodies.

METHODS. BALB/c mice were immunized with purified PilC_{His6} protein and a PilC1-specific peptide (provided by K. H. Wiesmüller) to produce the AK199 and AK200 antisera, respectively. Gonococci were suspended in PBS, adsorbed onto Formvar-coated copper grids and fixed with 2% paraformaldehyde in PBS. The grids were incubated in 1:100 dilutions of anti-PilC sera. Bound first antibodies were visualized with gold-conjugated anti-mouse IgG secondary antibodies (provided by H. Schwarz). After staining with 2% uranyl acetate for 3 min (a, b) and rotary shadowing (c, d), samples were viewed and photographed in a Zeiss M109 electron microscope at 80 kV.



find an approximately 20% reduction of gonococcal adherence to epithelial cells (data not shown). The weak inhibitory effect of the available antisera is not surprising, considering the likelihood of the pathogen adopting immune evasion strategies to protect its crucial adherence function. An understanding of the PilC structure will be essential for the development of a potent pilus-based vaccine. □

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A B-cell coactivator of octamer-binding transcription factors

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THE octamer motif (ATGCAAT) paradoxically plays a central role in mediating the activity of both B-cell specific and ubiquitous promoters. It has been widely assumed that the predominantly lymphoid-restricted octamer-binding factor Oct-2 mediates tissue-specific promoter activity, whereas the ubiquitously expressed Oct-1 mediates general promoter activity, but this view has been challenged¹⁻⁶. Here we use a modified yeast one-hybrid assay to isolate a B-cell factor, Bob1, which associates with either Oct-2 or Oct-1. In transfection experiments, this factor boosts Oct-1-mediated promoter activity and to a lesser extent, that of Oct-2. This coactivation is strictly dependent on the specific interaction with Oct-1 or Oct-2 because deletion of the octamer motif abolishes coactivation. We conclude that Bob1 could represent a new tissue-specific transcriptional coactivator which may convert a

ubiquitously expressed transcription factor to a cell-type-specific activator.

A modified yeast one-hybrid assay⁷ was used to identify proteins capable of interacting with human Oct-2A. Of ten strongly positive clones isolated from a GAL4 activation-domain-tagged human peripheral lymphocyte library⁸, one showed an absolute requirement for coexpressed Oct-2A to activate transcription (Fig. 1a). Analysis of this clone revealed a proline-rich and acidic putative open reading frame of 256 amino acids (Fig. 1b). This clone shares some sequence similarity with human TAF_{II} 250 (Fig. 1c).

We analysed the expression profile of this clone by northern blotting. A major band of 2.8 kilobases (kb) was predominantly expressed in human tissues of lymphoid origin, especially spleen, peripheral blood leukocytes and thymus (Fig. 2a). The same transcript was also observed in tissue of the small intestine, an organ known to contain cells of lymphoid origin (Peyer's patches). The cell-specific expression profile of the transcript was analysed by comparing three B-cell lines, two T-cell lines and an osteosarcoma line. We found the transcript was expressed exclusively in cells of B-cell origin (Fig. 2b). On the basis of its restricted expression pattern and binding properties, we named this clone Bob1 (B-cell Oct-binding protein-1).

To test the specificity of the Bob1/Oct-2 interaction, we analysed the formation of Bob1 protein complexes by bandshift experiments. A supershifted Oct-2 complex was observed in BJA-B nuclear extracts preincubated with *in vitro* translated Bob1 (Fig. 3a). Surprisingly we also observed a supershifted Oct-1 complex with these extracts. This was confirmed in band-

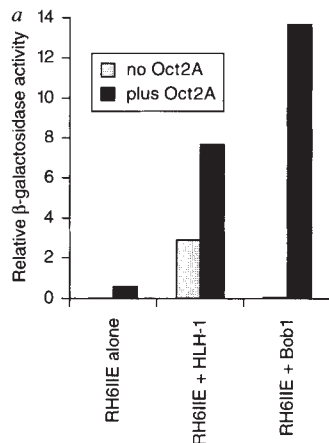


FIG. 1 a, Interaction of Bob1 and Oct-2A in yeast. A yeast strain RH-61E was constructed bearing two integrated reporter genes, *HIS3* and *lacZ*, which have had the yeast UAS-gal control sequences replaced by a 6× multimerized IgH intron enhancer submotif containing the octamer motif and E4 box site⁶. Full-length human Oct-2A¹⁴ was expressed constitutively in this strain from plasmid pASO2, a derivative of pAS1⁸. This strain, either expressing human Oct-2A or not, was transformed with Bob1 or HLH-1. RH-61E was transformed with the human lymphocyte library and positive clones isolated as described⁸. β-galactosidase was assayed as described¹⁵; relative levels of β-galactosidase activity in these strains are shown, normalized for protein content. b, Sequence of Bob1 (accession number, X83504). Nucleotide and deduced amino-acid sequence of a full-length Bob1 cDNA clone, clone 49, isolated from the human peripheral lymphocyte library used for the screen. The initial ATG is followed by an open reading frame of 768 bp encoding a proline-rich (16%) and acidic protein of 256 amino acids. *In vitro* transcription and translation of clone 49 gave a major polypeptide band on an SDS-polyacrylamide gel of the size predicted for Bob1. Asterisk indicates the in-frame stop codon. c, Sequence comparison between human TAF_{II} 250 and Bob1. Identities are marked by a bar and similarities by dots.

b

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1  CCCGGTCTCACATTAAGAAGCCAACTGTCGGCTTCAAGAGAAAAGGCAACATCTCTGT  60
61  CACAGGCCATGCTCTGGCAAAACCCACAGCTCCGGAGCAAGCCCGAGCCCGGCGCCG  120
    M L W Q K P T A P E Q A P A P A R P
121  CATACCAGGGCGTCCGTGTGAAGGAGCCAGTGAAGGAAGTCTGAGGAGGAAGCGAGGCC  180
    Y Q G V R V K E P V K E L L R R K R G H
181  ACGCCAGCAGTGGGGCAGCACCTGCACCTACGGCGGTGGTGTGCCCATCAGCCCTGG  240
    A S S G A A P A P T A V V L P H Q P L R
241  CGACCTACACCCAGTGGGTCTCTCTGCTGGACATGGAAGTTCTGTGTCTGCACTGA  300
    T Y T T V G P S C L D M E G S V S A V T
301  CAGAGGAGGCTGCCCTGTGTGTCGGGTGGCTTCCAGCCACCCCGGCCACCTGACGC  360
    E E A A L C A G W L S Q P T P A T L Q P
361  CCCTGGCCCCATGGACCTTACACCGAGTATGTGCCCATGAAGCTGTCACTGTCCTCT  420
    L A P W T P Y T E Y V P H E A V S C P Y
421  ACTCAGCTGACATGTATGTGACGGCCGTGTGCCCGAGCTACACGGTGTGGGGCCCTCT  480
    S A D M Y V Q P S C P S I S T V V G P S S
481  CAGTGTGTACCTATGCTCTCCGCACTCATCAACATGTACAGACAAGAAGCTCCGCCA  540
    V L T Y A S P P L I T N V T T R S S A T
541  CGCCCGCAGTGGGGCCCCGCTGGAGGCCAGAGCAGGACCCCTCACCTATTTC  600
    P A V G P P L E G P E H Q P T P A T L Y F P
601  CGTGGCTCAGCCCCCTTCCACACTACCCACCTCCACCTGCACTACCGCTCCGGGCC  660
    W P Q P L S T L P T S T L Q Y Q P P A P
661  CAGCCCTACCTGGGCCCCAGTTTGTCCAGCTCCCATCTCTATCCAGAGCCAGTCTTC  720
    A L P G P Q F V Q L P I S I P E P V L Q
721  AGGACATGGAAGACCCAGAGAGCCCGAGCTCGTTGACCATGACAAGCTGCTTTTGG  780
    D M E D P R R A A S S L T I D K L L E
781  AGGAAGAGGATAGCGACGCTATGCGCTTAACCACTCTCTCTGTGGAAGGCTTTTAGG  840
    E E D S D A Y A L N H T L S V E G F *
841  CGTGGCTCCCACTGAGTCTCTTCCCTGAACITGGGATTTTAAATGAGCTGGAATIG  900
901  AGCCCGAGGTCATGCTGTGTGGAGTAGTCATTTACACTACACTTCTACGCACAGC  960
961  TAGAATTGTAGACCTGTAACCTTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT  1012

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c

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Bob1      88  LSQPTPATLQPLAPWPTYTEYVPEHAVSCPSADMYVQVPCPSYTVVGPS  137
      |||||  .:|||  . . . . . :. . . . . :|||  . . . . .
TAFII250  628  LSQPGPHSVQPLKHKIKKAKMREQERQASGGGEMFMRPTQDLTKDKD  677
Bob1      138  SVLTYS...PPLITNV  151
      .:  |  .:|  .:|
TAFII250  678  LILAIEYSENGPLMMQV  694

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Searches were made of the SwissProt database with the FASTA program of the Genetics Computer Group GCG package¹⁶.

shifts of HeLa cell nuclear extracts which gave the same super-shifted Oct-1 complex (Fig. 3b).

Oct-1 and Oct-2 exhibit a striking degree (94%) of sequence identity in their DNA-binding POU-specific and POU homeo-domain regions, but have less similarity in regions outside this domain^{9,10}. Hence we reasoned that the POU domains of these two proteins could serve as a common interface for interactions

with Bob1. This was confirmed by the appearance of a Bob1 supershifted complex after preincubation with the Oct-1 POU domain alone (Fig. 3d). This interaction with POU domain proteins must be highly selective as Bob1 is unable to form a super-shifted complex with either N-Oct3 (Fig. 3c) or N-Oct2 (not shown), POU-domain proteins that are 74% identical to Oct-1 and Oct-2 in this region^{9,11}.

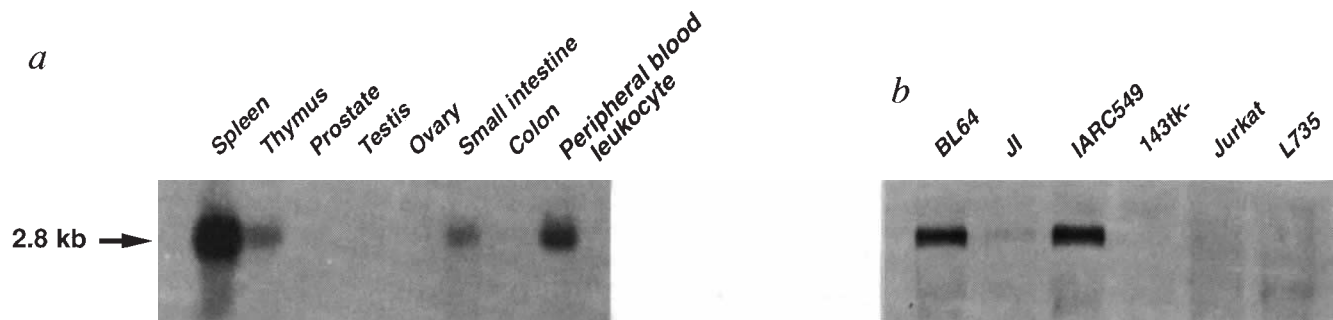


FIG. 2 Expression profile of Bob1. Northern blot analysis of Bob1 in *a*, different human tissues, and *b*, different human cell lines. Human cell lines are as follows: BL64 and JI, Burkitt lymphoma B-cell lines; IARC549, lymphoblastoid B-cell line; 143tk-, osteosarcoma line; Jurkat, T-cell line; L735, T-cell line. Further analysis of this transcript's expression profile indicated it was present in Namalwa and Rajii human Burkitt lymphoma cell lines, as well as in BJA-B, a human lymphoblastoid line, but was not present in HeLa, Chang liver cells, LN18 (glioma) or SK-N-BE (neuroblastoma) cell lines (data not shown).

METHODS. *a*, A human multiple tissue blot of poly(A)⁺ RNA (2 µg, Clontech) was hybridized with a random-primed ³²P-labelled Bob1 full-length cDNA according to the manufacturer's recommendations. *b*, Total RNA (20 µg) was electrophoresed through 1.2% agarose gels and transferred to nitrocellulose membranes using standard techniques¹⁷. The filter was probed with a full-length digoxigenin-labelled Bob1 riboprobe. Immunological detection with anti-digoxigenin-Fab (Boehringer Mannheim) was according to the manufacturer's instructions using CSPD chemiluminescent substrate (Tropix).

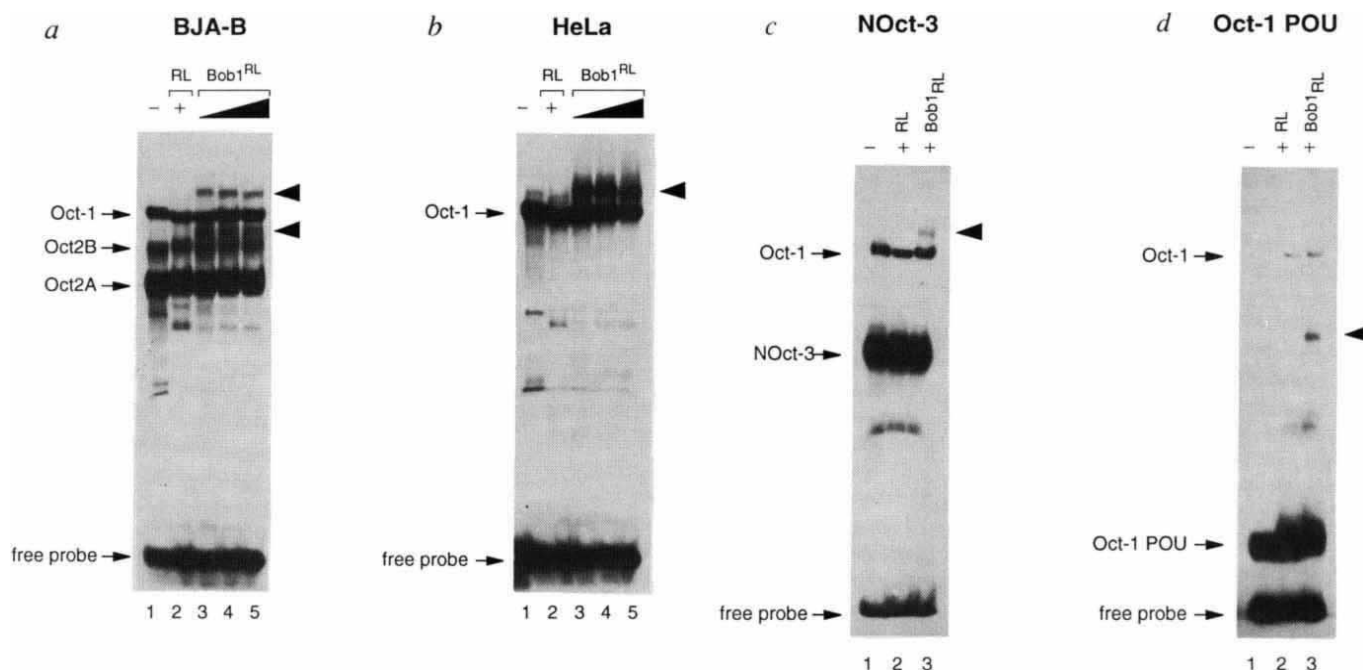


FIG. 3 Interaction of Bob1 with DNA-bound octamer factors *in vitro*. An octamer containing oligonucleotide of the Ig-κ promoter⁶ was used in electrophoretic mobility shift assay (EMSA) with extracts containing different octamer factors and FLAG-epitope-tagged Bob1 protein translated *in vitro* using rabbit reticulocyte lysates (Bob1^{RL}). Arrowheads on the right of the gels indicate supershifted complexes. *a*, EMSA with BJA-B nuclear extracts (2 µg). Lane 1, BJA-B extract alone; lane 2, BJA-B extract + rabbit reticulocyte lysate (RL) alone (6 µl); lane 3, BJA-B extract + Bob1^{RL} (3 µl); lane 4, BJA-B extract + Bob1^{RL} (6 µl); lane 5, BJA-B extract + Bob1^{RL} (10 µl). *b*, EMSA with HeLa nuclear extracts (5 µg); lane order as in *a*. *c*, EMSA with N-Oct3 expressing HeLa nuclear extracts (2 µg). Lane 1, nuclear extract; lane 2, nuclear extract + RL alone (4 µl) lane 3, nuclear extract + Bob1^{RL} (4 µl). *d*, EMSA using purified Oct-1 POU-domain protein (4 ng) expressed in *E. coli*¹⁸; lane order as in *c*. Preincubation of Oct-1 reaction mixtures with an anti-FLAG epitope Ab (IBI) gave a complex supershifted further, con-

firmed the presence of Bob1 in the supershifted complexes (data not shown).

METHODS. The indicated amounts of nuclear extracts, reticulocyte lysates and pure proteins were preincubated for 15 min at room temperature. After addition of the bandshift buffer¹¹ containing an end-labelled oligonucleotide (10 fmol) with an octamer site from the Ig-κ promoter⁶, the reaction mix was incubated for a further 20 min at room temperature before being loaded onto a 4% polyacrylamide (1:19) gel (0.25 × TBE). Nuclear extracts from HeLa cells were prepared as described⁶. Bob1 cDNA was transcribed *in vitro* from pCATCH, an N-terminally FLAG-epitope tagged expression vector (manuscript in preparation), and *in vitro* translated using rabbit reticulocyte lysate (Promega). N-Oct3 nuclear extracts were prepared from HeLa cells transfected with 3 µg per 100-mm dish of pEVRF0-NOct3 expressing full-length N-Oct3 (ref. 11) by the calcium phosphate co-precipitation method⁶. BJA-B nuclear extracts were prepared as described¹⁹.

We next investigated whether the specific interaction between either Oct-1 or Oct-2 and Bob1 could influence transcriptional activation mediated by these POU-domain regulatory factors by using S1 nuclease protection analysis of an immunoglobulin κ light-chain promoter reporter containing a single octamer site⁶ and co-transfection of Bob1 into a non-B-cell line. Co-transfection of a Bob1 expression vector in HeLa cells strongly stimulated transcription mediated by endogenous Oct-1 on this

promoter element, whereas co-transfection of vector alone had no effect (Fig. 4a, lanes 2 and 3). Expression of Oct-2A moderately stimulated this promoter, the effect being boosted by co-transfection with Bob1. This stimulation was strictly dependent on the presence of an octamer motif in the promoter element of the reporter because it was abolished when this element was deleted (Fig. 4b, lanes 1 and 2). This transcriptional coactivation was specific, as demonstrated by Bob1's inability to boost transcription mediated by another ubiquitously expressed transcription factor, Sp1 (Fig. 4b, lanes 3 and 4).

We observed similar effects with an IgH mini-enhancer element containing an octamer site in a promoter position (the same element used for the yeast screen). Here Bob1 co-transfection greatly stimulated transcription from this reporter compared with expression vector alone (Fig. 4c, lanes 2 and 3). Bob1 further boosted Oct-2-mediated transcription from this element (Fig. 4c, lanes 4 and 5).

The deduced amino-acid sequence of Bob1 is proline-rich and acidic, from which we inferred that Bob1 itself may have an intrinsic transcriptional activation domain similar to other proline-rich regulatory factors¹². We therefore co-transfected a GAL4(1-93) DNA-binding-domain-Bob1 chimera into HeLa cells together with a GAL4 DNA-binding-site reporter plasmid and found that the chimera strongly boosted transcription from this reporter compared to GAL4(1-93) alone (Fig. 4d, lanes 2 and 3). We conclude that Bob1 has inherent transcriptional activity and that its specific transcriptional coactivation function may be elicited by its selective tethering to appropriate promoter elements by Oct-1 or Oct-2. Our results explain why an absence³ or marked reduction⁵ of Oct-2 in B cells has no effect on the amount of endogenous immunoglobulin transcription, whereas mutation of the single octamer site in the promoter of these genes does¹³.

Bob1 thus represents a new and apparently cell-specific co-activator which can, by virtue of specific protein-protein interaction, confer cell-specific activation on an otherwise ubiquitously expressed transcription factor.

Note added in proof: While our work was in progress, Patrick Matthias (Friedrich Miescher Institute, Basel) together with Michel Strubin (University of Geneva) independently isolated, in a screen for proteins interacting with Oct-1, a cDNA encoding the same protein and designated it OBF-1 (ref. 21). Bob1 may also be related to the B-cell coactivator OCA-B described in ref. 1.

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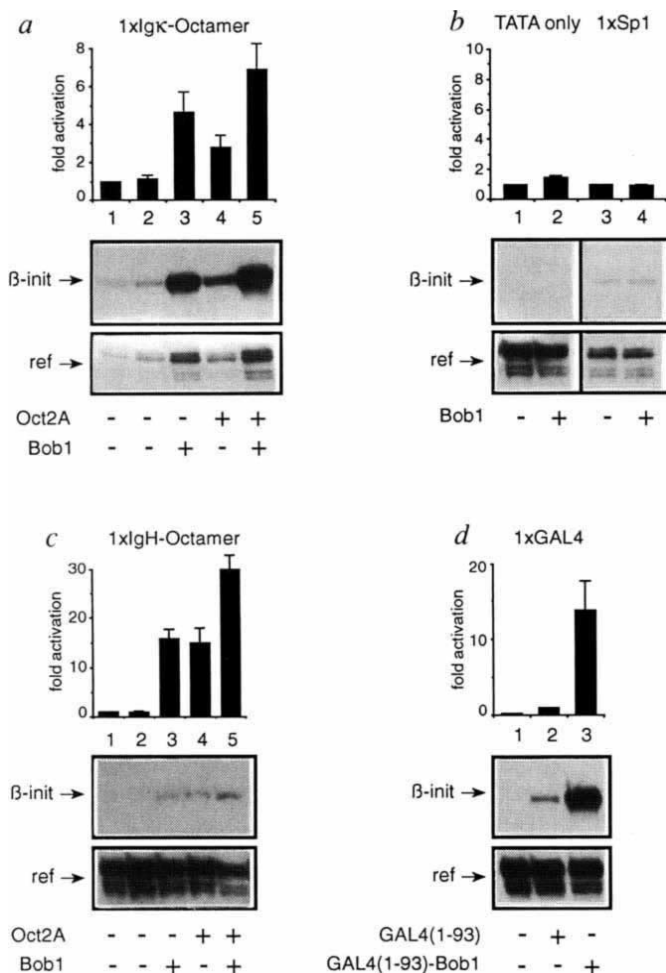


FIG. 4 Octamer-factor-specific coactivation of transcription by Bob1. HeLa cells were cotransfected with OVECS reporter plasmids⁶ containing the indicated promoter elements and different expression plasmids as described below and analysed for transcriptional activity. **a**, An Ig- κ -OVECS plasmid containing a single octamer-binding site⁶ was used as a reporter. Effector plasmids expressing Oct-2A¹⁴ (2 μ g) or FLAG-nuclear localization signal tagged full-length Bob1 (5 μ g) pCATCH-Bob1, were transfected as indicated (lanes 3-5), lane 2, empty pCATCH vector. **b**, The octamer site deletion reporter OVECS⁶ (5 μ g, lanes 1 and 2) and a plasmid with a single Sp1 site upstream of the TATA box of OVECS²⁰ (5 μ g; lanes 3 and 4) were used as reporters. 5 μ g pCATCH-Bob1 was transfected as indicated (lanes 2 and 4). **c**, 4 μ g of an OCTA-1⁶ reporter plasmid containing an IgH enhancer octamer site in a promoter position was cotransfected with expression vectors as in **a**. Lane 2, pCATCH vector alone. **d**, The plasmid 1GOVECS¹² with a single GAL4 binding site was used as reporter (5 μ g); 2 μ g effector plasmids expressing GAL4(1-93) or GAL4(1-93)-Bob1 were used as indicated. All transfections contained 0.5 μ g OVEC-REF⁶ plasmid for normalizing variability in transfection efficiency. 'β-Init' indicates the signal of tested reporters; 'ref' indicates the signal of the reference plasmid OVEC-REF. Average values of corrected reporter signals derived from transfections repeated 2-4 times are depicted. Standard errors are superimposed. Quantitative EMSA of extracts of transfected cells indicated that Bob1 cotransfection had no effect on either endogenous Oct-1 or co-transfected Oct-2 protein levels (data not shown).

A Rosetta Stone of mammalian genetics

Joseph H. Nadeau, Patricia L. Grant, Srinivas Mankala, Andrew H. Reiner, Joel E. Richardson and Janan T. Eppig

The Mammalian Comparative Database provides genetic maps of mammalian species. Comparative maps are valuable aids for predicting linkages, developing animal models and studying genome organization and evolution.

SINCE the earliest days of mammalian genetics, the possibility was recognized that many chromosomal segments were evolutionarily conserved in widely separated lineages¹. Initially, these data were modest in number and consisted exclusively of phenotypic comparisons of mutant genes such as those affecting coat colour. With time, more rigorous definitions of homologous genes were developed, largely because of the introduction of specific assays that provided for molecular comparisons, such as isozyme assays based on defined biochemical functions, and molecular techniques for assessing similarity through cross-hybridization and DNA sequence analysis. In addition, the amount and quality of information has increased rapidly, in part because of the success of the Human Genome Project². For example, the number of genetically mapped genes in the mouse genome has doubled from 2,598 to 5,518 in the past 30 months.

Several years ago, we began exploring various electronic formats to manage, display, and distribute comparative mapping information. The Mammalian Comparative Mapping Database was conceived as an information resource for documenting the existence of homologous genes and their chromosomal location among mammalian species. The emphasis of the database was placed on mammalian species because of our own research interests and because of the medical relevance of mammals as models for human genetic diseases. As data become more substantial and as understanding grows, new applications for comparative maps are being found, including linkage prediction, identification of animal models for human genetic diseases, and transfer of genome reagents and information from 'map-rich' to 'map-poor' species²⁻⁶. The Mammalian Comparative Mapping Database has evolved to become a component of the Mouse Genome Database (MGD)⁷.

Database contents

For each mammalian locus for which a homologous locus in another species has been defined, the database contains the following information for the species' homologue: species, gene or anonymous locus symbol, chromosomal assignment, regional localization (genetic distance from the centromere or cytological band assignment, currently for human homologues only), mouse locus symbol, mouse

chromosomal assignment and regional localization, and references documenting the mapping of these loci and the evidence for homology. For example, the database documents the homology of *Bcl2* 61.0 centimorgans from the centromere of mouse chromosome 1 and *BCL2* on human chromosome 18, band q21.3, together with appropriate references.

Mapping information is derived from numerous sources including published comparative mapping reports⁸, Mouse Chromosome Committee Reports⁹ and our weekly review of the primary literature. Standard guidelines recommended by the Comparative Mapping Committee are used to define homologous genes and identify homologous anonymous loci⁸. These are based on comparable biochemical properties in mouse *Glo1* and human *GLO1*, for example; on cross-hybridization in mouse *Anx4* and human *ANX4*; and on DNA sequence similarities in mouse *Rxb* and human *RXR*. Data validation is a high priority. Considerable effort is expended verifying all database entries.

A potential homology between a mouse and human gene would be verified by checking the citation for the purported homology and by confirming gene symbol, name and chromosomal localization in the locus mapping data component of MGD (for the mouse gene) and in the Genome Data Base, GDB (for the human gene). Additional validation, such as a DNA sequence comparison search, may be necessary in some cases. The relevance of novel disease models and the agricultural importance of mammalian species other than humans and mice is increasingly recognized⁶. The database therefore includes information for any mammalian species in which at least one gene or anonymous locus has been mapped and for which a homologue has been identified in another mammalian species. Currently, 41 species are represented (table right). The number of genes included for these species varies nearly 150-fold, from two for river buffalo and the Virginia opossum to 1,745 and 1,334 for humans and mice, respectively.

Database information is freely available in whole or in part for use in research publications and reports. The only condition is that proper acknowledgement of the information source should be included in any hard-copy or electronic publication. The recommended format is as follows: "Mammalian Comparative Mapping Data,

Loci that have homologues among mammalian species. The number of loci include only those loci for which a homologue has been identified in at least one other mammalian species

Species	Number of loci
antechinus, brown (<i>Antechinus stuartii</i>)	4
baboon (<i>Papio</i>)	48
buffalo, river (<i>Bubalus bubalis</i> L.)	2
cat (<i>Felis catus</i>)	60
chimpanzee (<i>Pan troglodytes</i>)	63
cow (<i>Bos taurus</i>)	240
deermouse (<i>Peromyscus maniculatus</i>)	23
dog (<i>Canis familiaris</i>)	39
dunnart, fat-tailed (<i>Smithopsis crassicaudata</i>)	10
echidna (<i>Echidna tachyglossus aculeatus</i>)	5
fox, silver (<i>Vulpes fulvus</i>)	8
gibbon (<i>Hylobates nomascus concolor</i>)	41
goat (<i>Capra hircus</i> L.)	8
gorilla (<i>Gorilla gorilla</i>)	42
hamster, Chinese (<i>Cricetus griseus</i>)	64
hamster, Syrian (<i>Mesocricetus auratus</i>)	3
horse (<i>Equus caballus</i>)	20
human (<i>Homo sapiens</i>)	1745
kangaroo, eastern grey (<i>Macropus giganteus</i>)	3
kangaroo, red (<i>Macropus rufus</i>)	10
lemur, mouse (<i>Microcebus murinus</i>)	36
marmoset, cotton topped (<i>Saquinus oedipus</i>)	25
mink, American (<i>Mustela vison</i>)	60
monkey, African green (<i>Cercopithecus aethiops</i>)	35
monkey, Capuchin (<i>Cebus capucinus</i>)	26
monkey, owl monkey	
monkey, owl monkey 1 (<i>Aotus trivirgatus</i>)	16
monkey, owl monkey 2 (<i>Aotus trivirgatus</i>)	29
monkey, owl monkey 3 (<i>Aotus trivirgatus</i>)	27
monkey, owl monkey 4 (<i>Aotus trivirgatus</i>)	26
monkey, owl monkey 5 (<i>Aotus trivirgatus</i>)	36
monkey, owl monkey 6 (<i>Aotus trivirgatus</i>)	55
monkey, owl monkey 7 (<i>Aotus trivirgatus</i>)	13
monkey, Rhesus (<i>Macaca mulatta</i>)	37
mouse, laboratory (<i>Mus musculus/domesticus</i>)	1334
opossum, brush-tailed (<i>Trichosurus vulpecula</i>)	16
opossum, Virginia (<i>Didelphis virginiana</i>)	2
orangutan (<i>Pongo pygmaeus</i>)	35
pig (<i>Sus scrofa domestica</i> L.)	103
planigale (<i>Planigale maculata</i>)	5
platypus (<i>Ornithorhynchus anatinus</i>)	21
rabbit (<i>Oryctolagus cuniculus</i>)	58
rat, laboratory (<i>Rattus norvegicus</i>)	335
rosamond antechinus (<i>Dasykaluta rosamondae</i>)	7
sheep (<i>Ovis aries</i>)	73
wallaby, red-necked (<i>Macropus rufogriseus</i>)	5
wallaby, tammar (<i>Macropus eugenii</i>)	27
wallaroo (<i>Macropus robustus</i>)	14

Mouse Genome Database, Mouse Genome Informatics Project, The Jackson Laboratory, Bar Harbor, Maine, World Wide Web (URL: <http://www.informatics.jax.org>), date."

Database design

The database documents the chromosomal location of homologous genes and anonymous loci among different mammalian species. We anticipate that as community-based mapping databases are developed for more mammalian species, the mammalian comparative mapping data in the MGD will evolve to take advantage of these resources. The database will need to store only the unique identifiers for the homologous loci in mammalian species and a table of pointers to other databases where the mapping data for those species will reside. For example, in the future, a mouse-human homology would be stored with a pointer to GDB for mapping data for the human gene; or a mouse-pig homology would be stored with a pointer to PIGBASE for mapping data for the pig gene. By storing electronic pointers to corresponding entries in other species-specific databases, the user will be provided with transparent access to the full gene or locus report in diverse databases. This mechanism takes advantage of the species' databases in maintaining nomenclature and current mapping data for that species and concentrates the efforts for the MGD mammalian comparative mapping data on maintaining current information on homologies between species. These pointers are based on identification of the corresponding Unique Identifiers for each homologous gene or locus in each database.

Access

The Mouse Genome Database, which contains the mammalian comparative mapping data, is available on the World Wide Web (WWW). Anyone with access to the Internet and with appropriate browsing software can connect to the Bioinformatics Server of The Jackson Laboratory on the WWW (the URL address is <http://www.informatics.jax.org>).

Once connected, querying the data is easily accomplished by entering search parameters into a form. Further navigation is accomplished by point-and-click for those with graphic terminals and by cursor actions for those with character-cell terminals. The comparative mapping data in MGD are viewed as tables that relate mouse loci to homologous loci in other mammals (Fig. 1).

In addition, interactive graphical map displays can be obtained through the Encyclopedia of the Mouse Genome, a software tool for displaying genetic maps and associated information developed by the Mouse Genome Informatics Group at

mammalian species (a 'species-on-mouse' map). Selecting a specific locus on these maps or from the locus list causes the reference and note window to respond accordingly, thus providing the user with appropriate information for that locus and the defined homology. The Encyclopedia software is available in both UNIX and Macintosh versions via anonymous file transfer protocol (ftp) at the address <ftp.informatics.jax.org> or by contacting User Support (email: mgi-help@informatics.jax.org). Once the Encyclopedia is installed on the user's computer, one may then obtain maps live from the MGD through the WWW. The WWW browser can be configured to automatically start the Encyclopedia when a map is retrieved. For more information, contact User Support.

Maps

Graphic displays are frequently easier to comprehend, and they convey more information in a specific context than can textual data. The graphical comparative maps we have developed to display homologous loci between mammalian species use the mouse as the reference map or underlying map (Fig. 2, ref. 10). To use a species map as the reference map, that species must have a well-developed genetic map. Aside from the mouse, the human map is the only other mammalian species that currently has a sufficiently defined linkage map to be suitable for use as the reference species for a comparative map display. Comparative maps that display loci from a second species superimposed on the human map are under development. Figure 2 shows a comparative map that utilizes the Encyclopedia of the Mouse Genome software as its display tool. Comparative maps can also be printed from files translated into postscript using an e-mail Map-Draw utility we developed¹¹.

Mapping data that establishes the locations for mouse genes and anonymous loci are derived from the Mouse Chromosome Committee Reports⁹ (Fig. 2) or from MGD which includes Chromosome Committee Report localizations plus additional data derived from the

The screenshot shows a web browser window titled "Mouse Genome Database: Mammalian Homology Data". The page displays "Query Results - Summary" for a search. The results are organized into sections based on gene names starting with 'a'. Each section contains a table with columns: Gene, Species, Symbol, Chrom, and Reference(s). The loci listed include: adrenergic receptor kinase, beta-1; adrenergic receptor, alpha-2A; adrenergic receptor, beta-1; aldehyde dehydrogenase-2, cytoplasmic; and antigens identified by monoclonal antibodies 4F2. Each entry provides the species (human, mouse, laboratory, cow, chimpanzee), the locus symbol (e.g., ADRA1, ADRA2, ADRA3, ALDH2, AID1), the chromosome (19), and a reference to a scientific publication.

Gene	Species	Symbol	Chrom	Reference(s)
adrenergic receptor kinase, beta-1				
human		ADRA1	11q13	Petrovic JL, FBB Lett 283:122-6, 1991
mouse, laboratory		Mrbk1	19	Petrovic JL, J Biol Chem 266:14929-46, 1991
adrenergic receptor, alpha-2A				
cow		ADRA2	26	Therrien JJ, Crit Crim Cell Genet 37:123-126, 1991
human		ADRA3	10q24-q26	Yano-Tsuo TK, Proc Natl Acad Sci U S A 87:1516-20, 1990
mouse, laboratory		Mrbk2	19	Gibby BJ, Genomics 18:329-44, 1991 Yano-Tsuo TK, Proc Natl Acad Sci U S A 87:1516-20, 1990
adrenergic receptor, beta-1				
human		ADRA1	10q24-q26	Yano-Tsuo TK, Proc Natl Acad Sci U S A 87:1516-20, 1990
mouse, laboratory		Mrbk1	19	Gibby BJ, Genomics 18:329-44, 1991 Yano-Tsuo TK, Proc Natl Acad Sci U S A 87:1516-20, 1990
aldehyde dehydrogenase-2, cytoplasmic				
cow		ALDH2	9	Therrien JJ, Genomics 5:22-8, 1990 Therrien JJ, Crit Crim Cell Genet 31:1091, 1990
human		ALDH2	9p21	Wu LC, Am J Hum Genet 38:641-8, 1986 Sapich-Ginsberg L, Genomics 2:267-9, 1990
mouse, laboratory		Ald2	19	Tines DP, Genomics 27:307-36, 1991 Tines DP, Mouse News Lett 66:68, 1990
antigens identified by monoclonal antibodies 4F2				
human		AID1	11q12.1-q13.1	Goodfellow PM, Crit Crim Cell Genet 37:481, 1990
mouse, laboratory		Mrbk1	19	Zochelle JM, Genomics 14:26-31, 1992
chimpanzee		AID1	9	Jones C, Crit Crim Cell Genet 48:662, 1990

FIG. 1 Five mouse Chromosome 19 loci with homologies in other mammals and with locus names beginning with 'a' were found (adrenergic receptor kinase, β -1, adrenergic receptor, α -2A, adrenergic receptor, β -1, aldehyde dehydrogenase-2, cytoplasmic, and antigen identified by monoclonal antibodies 4F2). For each locus name, the species, the locus symbol for that species, the chromosomal location of the gene and references are provided. In addition, each item underlined represents a hypertext link to additional information. By selecting a human locus symbol, for example, the user is immediately transferred to the human database (GDB) at the Johns Hopkins University and the results of querying for the locus selected are displayed. By selecting a mouse locus symbol, the user is taken to the locus data in the Mouse Genome Database (MGD). By selecting a reference, the user is taken to the MGD full reference information screen. The database can be traversed through many screens of related data by following these hypertext links.

The Jackson Laboratory (Fig. 2). These comparative maps display a mouse chromosome (genetic map) upon which are superimposed loci and map locations for homologous genes from a selected

anonymous loci are derived from the Mouse Chromosome Committee Reports⁹ (Fig. 2) or from MGD which includes Chromosome Committee Report localizations plus additional data derived from the

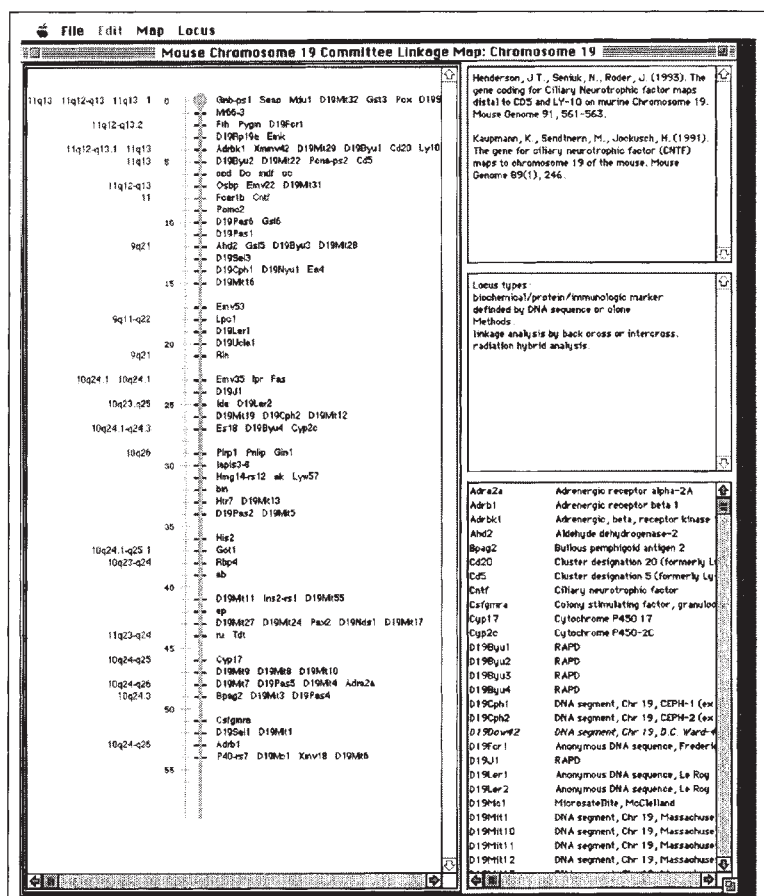


FIG 2 Mouse Chromosome 19 map displaying human homologies viewed using the Encyclopedia of the Mouse Genome software tool. To the right of the chromosome graphic are the mouse loci shown at their location on the chromosome. To the left are the locations of the homologous loci in humans. The map can be scrolled vertically and horizontally. The map may be magnified to view regions of high locus density in detail. In the tiled windows to the right

Frederick map², and from weekly review of the primary literature. Each gene or anonymous locus is displayed at its appropriate location. If a gene or anonymous locus has been assigned to a chromosome but its genetic or cytogenetic location has not been determined, it is displayed either as an italicized symbol in the locus-list window (for Encyclopedia display, see D19Dcw42 in Fig. 2) or near the Q arm telomere (bottom) of the chromosome¹⁰.

The future

Several enhancements to the map, to the database, and to the ability of the user to interact with these components are planned. First, a new feature will include the option to choose another species for the reference or underlying map display, for example, humans or cow. Using the human map as the reference map will be accomplished first; for many other species, mapping data remain too sparse to generate a good background reference map. Second, an enhancement to the data

of the chromosome graphic window is a reference window showing the references relevant to the locus selected (in this case *Cntf*), a note window with additional information of interest, and a list of all loci contained in the map that is being viewed. Note that the heading for this screen is 'Mouse Chromosome 19 Committee Linkage Map' indicating that the data source for this map is the 1994 Mouse Chromosome Committee Report.

will distinguish two kinds of references, namely those that establish homology and those that document genomic localization. Third, closer integration with other databases, such as protein and sequence databases, is anticipated. Fourth, and extremely important, is integration with databases that manage information resources for mammalian species other than mouse and human. And, finally, we encourage direct editorial input from scientists interested in the genetic maps for various mammalian species. We are eager to incorporate direct data submission methods as we have for the mouse genetic linkage component of MGD. Any individuals interested in representing a community working on one of the mammalian species included in the database should contact JTE (jte@informatics.jax.org).

User support

Questions, problems, concerns and suggestions should be communicated to User Support, the Mouse Genome

Informatics Project, The Jackson Laboratory, either by email addressed to mgi-help@informatics.jax.org or by telephone (207) 288-3371, ext. 1900 or by fax (207) 288-2516.

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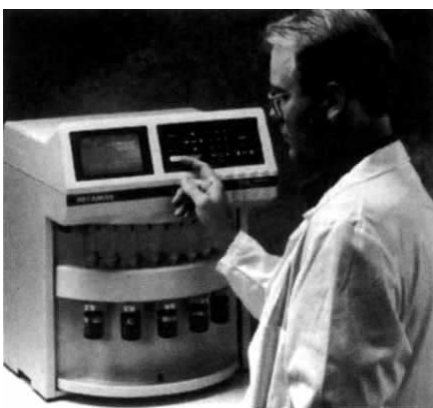
Reader Service No.3

Nucleic acid update

Fulfil your research needs and wants with an electroporation system, somatic cell hybrid panels, a manual DNA sequencing kit and a biospectrometry workstation.

EPICENTRE Technologies' **T4 RNA ligase** for production of full-length cDNA can be used on a wide range of substrates including RNA, DNA, oligo-ribonucleotides, oligo-deoxynucleotides and numerous derivatives (*Reader Service No. 101*). It can be used to facilitate the synthesis of full-length cDNA by ligation of oligonucleotides to the 5' and/or 3' ends of transcripts, allowing amplification of the entire transcript. The enzyme may be also used for 5' and 3' end-mapping and sequencing of RNAs, intra- and intermolecular RNA ligations, and in the construction of unique RNA and DNA-containing oligonucleotides. This enzyme is highly purified and free of contaminating nuclease activity.

For individuals or groups, Beckman has introduced its new **Oligo 1000M DNA synthesizer** (*Reader Service No. 102*). Designed for high-volume oligonucleotide needs, this synthesizer has 2-min cycle times and 10 min cleavage and deprotec-



The Beckman Oligo 1000M

tion. The manufacturer states that its efficient reagent consumption considerably lowers the cost of making oligonucleotides. Beckman is an ISO 9000 quality systems company.

BIOS Laboratories now offers two independent **somatic cell hybrid panels** for chromosome localization (*Reader Service No. 103*). There is a PSC hybrid panel (a polychromosomal panel) and a new MSC hybrid panel (a monochromosomal panel). Fluorescence *in situ* hybridization is also offered if sub-localization and confirmation is needed. The MSC panel consist of 24 cell lines, 22 of which contain a single human chromosome while the other two cell lines contain only two human chromosomes. This panel will provide an independent confirmation

of chromosome location and DNA for the generation of chromosome-specific libraries and probes. The manufacturer states that these panels make multi-gene families easier to identify and localize with more consistent and reliable results.

Bio-Rad Laboratories has recently introduced the new Gene Pulser II **electroporation system** (*Reader Service No. 104*). This apparatus offers electroporation to a wide variety of cell types. The core of the unit utilizes circuitry for pulse reproducibility and sample monitoring based on the actual pulse delivered is provided. For bacterial or yeast transformation Gene Pulsers' pulse controller offers increased



Bio-Rad Gene Pulser II

control of high-voltage pulses. Controlled resistance from 100–1,000 Ω in 100- Ω increments assists with the reproducibility of standard prokaryotic protocols. This unit uses a wide range of precision capacitors (25–3,275 μ F in 25- μ F increments) for electroporation of mammalian cells, sensitive eukaryotic cells and plant protoplasts

New England Biolabs has introduced Litmus **cloning vectors** for cloning, mutagenesis and *in vitro* transcription (*Reader Service No. 105*). These phagemid vectors feature universal sequencing primer sites, a streamlined pUC-derived plasmid backbone, M13 origin for single-stranded mutagenesis template production, blue/white selection, ampicillin resistance and polylinkers that contain a total of 32 restriction sites representing all commercially available four-base extensions. Litmus also contains a system for generating RNA probes in either direction using only T7 RNA polymerase.

■ DNA amplification

Pan Vera's TaKaRa LA PCR kit is intended for efficient **amplification of genomic DNA templates** greater than 5 kb (*Reader Service No. 106*). The LA PCR kit

provides the reagents necessary for amplification of large DNA templates. The manufacturer states that by combining an optimized buffer system with TaKaRa's Ex Taq polymerase, the kit provides routine extensions up to 20 kb. Amplification of larger products is demonstrated by the inclusion of a 21 kb control template and specific primers. This kit achieves fidelity by using an effective 3'–5' exonuclease activity. A dNTP mix and molecular weight markers are also included.

Stratagene has announced it is now a licensed source of enzymes used in the polymerase chain reaction process (*Reader Service No. 107*). The company offers **Pfu DNA polymerase** which is designed for high accuracy PCR or for when truly blunt-ended PCR fragments are desired. Stratagene also supplies Taq DNA polymerase and a range of PCR-related products including vectors for directional cloning and subcloning from ampicillin-resistant plasmids, polymerase enhancer for better yields and specificity, a buffer kit for optimization and an their Taq Extender PCR additive which extends the company's PCR for amplifying tough templates greater than ten kilobases in length.

■ DNA Probes

Ambion's new **S1-Assay kit** may be of special interest to those quantifying mRNA, doing 5' end-mapping of transcriptional start sites or other gene characterization studies (*Reader Service No. 108*). This kit can also be used to identify 5' and 3' ends of transcripts as well as the positions of introns within the gene encoding the target message. This assay accepts either RNA or DNA probes and provides researchers with an S1 nuclease and buffers that have already been standardized — eliminating time or reagents wasted in re-optimizing each new lot of enzyme.

Alpha Laboratories offers a full range of **cancer and cytogenetic probes** for *in situ* hybridization (*Reader Service No. 109*). The three major types of human satellite DNA are included: alpha, beta and the classical satellites. The range covers probes for microdeletion syndromes such as Smith-Magenis, Di George, Prader-Willi and Kallman syndromes. There is also an expanding number of whole chromosome paint probes. These probes are available together with kits for fluorescence or light microscopy

for fixed tissue, whole cells or metaphase spreads.

The Rad-Free Southern kit, from Schleicher & Schuell, is a non-enzymatic and non-radioactive **probe labelling and detection kit** for DNA, RNA and oligonucleotides (*Reader Service No. 110*). This

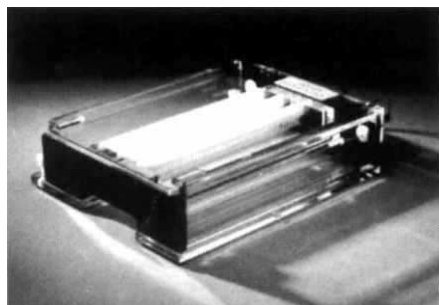


Southern kit

probe labelling system utilizes the photoreactive psoralen-biotin reagent and is designed to eliminate the variability associated with enzymatic reactions like random priming or nick translation. The stated sensitivity is in the sub-picomole range with exposure times of 1 h or less. Subsequent detection using the Rad-Free system is performed either by chemiluminescent detection using its chemiluminescent sheets or by calorimetric detection using BCIP/NBT substrate tablets. Rad-Free is said to be scaleable, to have a shelf life of about a year and to be capable of labelling agarose gels.

■ Purification

Owl Scientific has announced the new Centipede wide-format horizontal **electrophoresis system** (*Reader Service No. 111*). This system is designed to screen multiple samples — up to 150 may be



Centipede for gel electrophoresis

separated simultaneously on one gel. Using the system's microtitre-format combs with a multi-channel pipette, samples may be loaded directly from a 96-well plate following sample amplification. Centipede uses Owl's Easy-Cast design which provides a leak-proof seal without taping. The gel tray and lid are constructed of ultraviolet transmissible acrylic to allow sample viewing during gel run.

The PolyATtract 9600 series **mRNA isolation system** uses a 96-pin multi-magnet for the rapid isolation of mRNA directly from tissue or cells in a 96-well format (*Reader Service No. 112*). Simultaneous purification of up to 96 biological samples from cells or tissues to mRNA can be completed in about 2 h. This system is intended for cDNA production for such applications as amplification and

amplification-based cloning and detection experiments. To ensure success of downstream applications, it is recommended that this system be used with previously optimized procedures. For effective isolation of mRNA from small amounts of tissue (<2.5 mg) or culture cells (<10⁵ cells) this system focuses on effective disruption of cells, denaturation of nucleoprotein complexes, inactivation of endogenous RNase and purification of RNA from contaminating DNA and protein.

Pharmacia Biotech has upgraded its ImageMaster **electrophoresis evaluation system** with the new, high-resolution Sharp JX-325 scanner and with improved software that now includes a separate program for more features and a wider range of support and input functions (*Reader Service No. 113*). This PC-based



Pharmacia Image Master

electrophoresis evaluation system is used to capture, analyse and present one- (1-D) and two-dimensional (2-D) data with a choice of separate 1-D or 1-D/2-D application programs. The Sharp scanner has a maximum resolution of 236 dots per cm to detect and quantify bands from high-resolution electrophoresis techniques. The new software includes functions for analysing light bands on dark backgrounds and overlaying absorbance profiles.

The PlasmidFast Purification kit, from Amresco, is aimed at **plasmid purification** — 2 µg to 10 µg of plasmid from 1 ml of overnight culture in 30 min (*Reader Service No. 114*). This kit purifies plasmid DNA without the use of phenol, chloroform or purification resins. DNA purified with PlasmidFast is said to be suitable for enzyme digestion, sequencing or transformation and subcloning. Unlike using glass resins there are no fines to carry over. Each kit contains sufficient reagents for purification of DNA from one hundred 1-ml overnight cultures. This method does not require high-speed centrifugation, vacuum manifolds or organic disposal. The manufacturer states that this kit is scaleable and that quick through-put makes PlasmidFast a useful choice for screening large numbers of recombinants.

For **PCR-ready DNA** from human whole blood there is the Split Second DNA

preparation kit by Boehringer Mannheim (*Reader Service No. 115*). According to the company Split Second samples are ready in less than 30 min and using a procedure that involves only ten steps. All preparation takes place in a single microcentrifuge tube to maximize safety by limiting exposure to infectious agents. The kit eliminates hazardous phenol/chloroform extractions, lymphocyte separation gradients and tedious ethanol precipitations. There are enough reagents for preparing 50–250 blood samples for PCR. This product is intended for simultaneous processing of large numbers of human whole blood samples for PCR.

New from the Nest Group is their E²B **endotoxin extraction buffer** (*Reader Service No. 116*). This product is designed for efficient removal of endotoxins from nucleic acid preparations. E²B is intended for use during the purification of plasmids and other DNAs rather than after the preparations have been completed. It is used simply as an additional column wash after the prescribed washes have been completed. The company states that E²B can reduce the level of endotoxins by more than 100-fold to a level below 50 EU ml⁻¹. DNA from such preparations is said to be suitable for gene therapy, transgenic studies, and for transfection into even the most sensitive cell lines.

Stratatech Scientific has introduced the Floraclean **plant DNA extraction kit** for genomic DNA free from mitochondrial and chloroplast DNA (*Reader Service No. 117*). This kit can be used with a variety of plant types and does not use phenol or chloroform. The kit first isolates plant cell nuclei, thus eliminating the problems associated with high polysaccharide contamination. Floraclean is available in 10-, 25- and 100-preparation sizes.

■ Nucleic acid sequencing

Perkin-Elmer has introduced the Ampli Cycle sequencing kit for **manual DNA sequencing** (*Reader Service No. 118*). This kit is said to yield high quality, reproducible sequencing ladders from a wide variety of DNA templates, including PCR products. The AmpliCycle kit contains AmpliTaq DNA polymerase, a recombinant, thermostable polymerase from a modified *Taq* DNA polymerase gene in *E. coli*. This polymerase enzyme lacks 3'–5' and 5'–3' exonuclease activities, can support direct incorporation of labels or end-labelling of ³²P, ³³P and ³⁵S sequencing primers. In addition to DNA polymerase, the kit contains, dNTPs, ddNTPs, control template DNA and primers.

Hitachi software has announced MacDNASIS 3.5 accelerated **sequence analysis software** for Power Macintosh computers (*Reader Service No. 119*).

Performance enhancement in DNA and protein sequence analysis is stated as orders-of-magnitude times faster than the version that operated in PowerPC emulation mode. This has led to the acceleration of an array of sequence analysis functions including RNA secondary structure prediction, homology searching, and multiple sequence alignment. MacDNASIS 3.5 allows the user to access genetic information on CD-ROM and CD-DATA, which includes GenBank, EMBL, NBRF-PIR, SWISS-PROT and Prosite databases. This software provides enhanced colour graphics and the tools necessary to predict structures and interpret experimental results. It can also produce simulations of secondary structure, fragment assemblies, homologies and protein expression. Other features include: automatic multiple sequence alignment with phylogenetic trees, DNA motif searching, mutation site analysis, primer design and plasmid map drawing.

■ Instrumental analysis

The Voyager biospectrometry benchtop workstation with linear mass analyser from Perseptive Biosystems is an integrated system that is designed for the determination of accurate molecular weights on sub-picomolar quantities of proteins and other large biomolecules (*Reader Service No. 120*). This matrix-assisted, laser desorption ionization, time-of-flight mass spectrometer is intended for high-throughput and routine use by laboratory personnel. The Voyager system uses variable ion acceleration energy up to 30 kV, a 1,000-step, fully variable laser intensity and the 337 nm ultraviolet nitrogen laser which produces 3-ns pulses at repetition rates to 20 Hz. The data system uses a 486/33 IBM-compatible PC with 8 Mb RAM and 400 Mb hard-disk drive. Perseptive Biosystems Voyager



The Voyager MALDI-TOF spectrometer

system is run on a Windows-based graphical interface for biospectrometer control and data analysis.

New from Ultra Violet Products is ImageStore, a networking version of its GDS7500 gel documentation and analysis system. (*Reader Service No. 121*). This new networked system for IBM compatible or Apple Macintosh computers, allows researchers to send images directly to a workstation within a computer network. Consequently, image



UVP's GDS7500 network system

analysis can now proceed at the terminal while the system is free for other users. The ImageStore system is comprised of a high-resolution digital charge-coupled device camera with zoom facility, a stand, a colour monitor and a thermal printer.

Kinetic Imaging offers new features for its Komet software for quantifying DNA damage in cells (*Reader Service No. 122*). A very sensitive, single-cell gel electrophoresis technique, the comet assay detects DNA damage and repair in cells. Its rapidly increasing range of applications includes genotoxicity detection, biomonitoring, radiation sensitivity and the assessment of environmental pollution. The company states that it has enhanced Komet's image analysis which allows more detailed measurements of the comet, including distributed tail-movement analysis. Thresholds for head and tail discrimination are now separate and an image overlay showing the head and tail position simplifies analysis. This system identifies tail breaks and compensates measurements accordingly.

■ Labware

Belco Glass has introduced AutoBlot, its new miniature hybridization oven (*Reader Service No. 123*). This product is designed to free counter space and operate as a safe, convenient platform for DNA-DNA or DNA-RNA hybridization. This oven features a removable, six-position rotisserie, variable speed control, microprocessor control with digital set point, temperature display, user-initiated auto-tune PID constants and prompting controller. AutoBlot can be stacked so that two ovens may occupy the same footprint as one.

Labsystems has introduced a new family of Multiskan readers (*Reader Service No. 124*). The Multiskan RC is fully computer-controlled where as the Multiskan MS comes with built-in software. The RC system options include up to eight filters, optics for visible or UV light and an agglutination measurement system. A specially designed printer, additional cutoff and/or cubic spline program are available with Multiskan MS.

For secure dispensing of radionucleotides, Amersham Life Science has introduced the Redivue isotope dispenser (*Reader Service No. 125*). This device has three user-selected security levels allowing access to be tailored to individual



Amersham isotope dispenser

laboratory requirements. Data and dispensing details are automatically recorded in the dispenser's memory, thus enabling reliable monitoring and precise control of nucleotide usage. The stored data can be downloaded to a computer or printer to provide a permanent record of all dispensing operations providing a permanent record of all dispensing operations. Redivue nucleotides are stable at ambient temperatures due to a patented dye-stabilizer formulation. □

These notes are compiled by Brendan Horton from information provided by the manufacturers. For more details, fill in the reader service card bound inside the journal.

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